ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator Drs Glass, Hug, Stoll (for Bram Wakefield) Trainee Investigator (if any) _____

Application No 81-053 (P) Supporting Agency (if Non-ICDDR,B) _____

Title of Study Fasciolopsis Enteritis Adenovirus in Diarrhea

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 (Yes) No
4. Are subjects clearly informed about: As part of Surveillance Study
   (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 NA
   (e) Benefits to be derived Yes No NA
   (f) Right to refuse to participate or to withdraw from study Yes No NA
   (g) Confidential handling of data Yes No NA
   (h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure Yes No NA
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.

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

Principal Investigator

Trainee

87-053(P)
Rind: 18.12.87

SECTION I - RESEARCH PROTOCOL

1. Title : Determination of the occurrence of different fastidious enteric adenoviruses (FEAs) and an evaluation of their pathogenic role in viral diarrhoea in Bangladesh

2. Investigators - Sweden : Dr Goran Wadell, Department of Virology, University of Umea

Co-Investigator - Dacca : Dr Roger I. Glass
Dr M.I. Huq
Dr B.J. Stoll

3. Starting Date : December 1, 1981

4. Completion Date : December 1, 1982

5. Total Direct Cost : US$ 2500.00 (Laboratory costs to be absorbed by Dr Wadell through other sources)

6. Scientific Program Head :

This Protocol has been approved by the DTWG Working Group.

Signature of the Scientific Program Head: Islam

Date: 9/12/81

7. Abstract Summary:

The fastidious enteric adenoviruses (FEAs) characterized by their poor cultivatibility have recently been recognized as causative agents in viral diarrhoea. We plan to examine their frequency in 3 populations, a) patients with diarrhoea systematically selected from the Dacca hospital surveillance system; b) patients with diarrhoea from whom no organism is identified

c) a community control group of healthy family contacts of patients with non-adenovirus diarrhoea. If FEAs' are found to be prevalent or important from these phase 1 studies, laboratory methods will be brought to Dacca in order to pursue more extensive clinical and epidemiologic studies in the field.

8. Reviews:

a. Research Involving Human Subjects: _____

b. Research Review Committee: _____

c. Director: _____

d. BMRC: _____

e. Controller/Administrator: _____

SECTION II - RESEARCH PLAN

A. INTRODUCTION

1. Objective:

Fastidious enteric adenoviruses (FEAs), characterized by their poor in vitro cultivatibility, have recently been recognized as causative agents in viral diarrhoea.

Our objectives are:

1. to determine the number of FEA serotypes, establish the degree of genetic relatedness between FEA and recognized adenoviruses; to analyze the genetic variability within each FEA; to determine the frequency and distribution of the various FEA's in industrialized and developing countries and to develop simple methods for direct identification of different FEA's (Dr Wadell).

2. To evaluate the prevalence of FEAs among. 1) a random sample of patients with diarrhoea attending the Dacca hospital of the ICDDR,B. 2) a sample of patients from whom no agent can be found after testing for the routine pathogens (in V. cholerae, ETEC, Shigella, Salmonella, Campylobacter, rotavirus) and 3) a ccomunity control group of healthy family contacts of patients with non-adenovirus diarrhoea.

2. Background:

In the third world 80% of the rural population is without access to clean water. Furthermore, 85% of the population lack adequate excreta disposal facilities. Among children less than 5 years of age the diarrhoea episodes have been estimated to more than 500 million per year (barua, 1980). In certain regions the impact of the diarrhoeas are higher than in others. Indonesian children in the age of 0-2 years experience up to 20 diarrhoea episodes. In Latin America it has been estimated that children experience diarrhoea during 2 months per year. Finally in Bangladesh children below age of 5 experience about 4,5 diarrhoeal episodes per year, while adults experience about one diarrhoeal episode each year (RE Black, 1981).

Diarrhoeas in children have been estimated to cause 5 million deaths each year making it a leading cause of childhood mortality particularly in the developing world. Equally important is the fact that diarrhoea leads to subnormal weight gain - the original growth curve is frequently not reached before another diarrhoeal episode sets in. It has been estimated that only 40% of children with diarrhoeas have a satisfactory weight gain.

Attempts to identify the etiology of diarrhoeas can be successful in up to 83% of the cases (Stoll 1981). It is estimated

that diarrhoeas can be caused by bacteria, viruses and protozoa.

A major share of all infantile diarrhoeas are associated with the following viruses: Rotavirus, 27 nm particles - represented by Norwalk agent, Astrovirus, Calicivirus, Coronaviruses and Adenoviruses. Of these the three different serotypes of rota viruses are by far the most important. In a comparative WHO-study of diarrhoea, stool specimens from children below 5 years of age from 11 countries were analyzed. Rotaviruses were found in 27% of these. However, the portion of rotavirus positive specimens varied between different countries from 7 to 70%.

The 27 nm particles represented by Norwalk agent, include at least four serotypes; Norwalk agent, the related Montogomery county agent, Hawaii agent, Ditchling agent and the Cockle agent from London. These agents are frequently associated with outbreaks in schools, summer camps and other adolescent or adult populations. The prevalence of antibodies against Norwalk agent has been reported to be 50% in the fifth decade. The 27 nm particles thus seem to be rare causes of diarrhoea among infants.

The prevalence of antibodies against astrovirus is high and astroviruses may occasionally cause a mild form

of diarrhoea. The majority of the infections are subclinical (Lee and Kurtz). The astroviruses are prevalent in many species but do not cause diarrhoea in animals.

Outbreaks of "winter vomiting disease" (Cubitt et al). (1979) and diarrhoea among infants has been reported to be caused by calici viruses (Chiba et al. 1979).

Corona viruses represent a problem of identification in the electron microscope since stools frequently contain beaded membranes, which could be mistaken for corona virus particles. However, at least three outbreaks of diarrhoea associated with corona viruses have been reported. (Caul and Eggleston, 1977).

Adenoviruses will be presented in greater detail since one of the purposes of the proposed study is to acertain their etiological role in diarrhoeas. The human adenoviruses cause respiratory symptoms or eye diseases. 37 serotypes have been recognized and grown in tissue culture (Wigand et al. 1980, de Jong et al. 1981).

The adenoviruses are divided in 6 subgroups (Wadell 1979, Green et al. 1979, Wadell 1980). Among members of subgroup A (Ad 31), B (adenoviruses types 3 and 7) can be associated with outbreaks of diarrhoea frequently in asociation

with respiratory disease. (Kjellen et al. 1957). Furthermore members of subgroup C (Ad 1, Ad 2 and Ad 5) usually infect young children and cause respiratory disease. The infections then become persistent and viruses can be intermittently shed in stools for more than 3 years (Fox et al. 1969, Fox et al. 1977).

In 1975 Flewett et al. reported an outbreak of diarrhoea where the association to adenoviruses was detected by the electromicroscope. Adenovirus particles appeared in large amounts but could not be cultivated in vitro. It was suggested that these viruses could be variants of adenovirus type 7. Adenoviruses which could not grow in vitro was also detected by EM in stools from black infants in South Africa with acute summer gastroenteritis (Schoub et al. 1975). In an extensive EM study from Toronto on children with diarrhoea, adenoviruses were detected in 15% of sick children. Out of these 45% could not be grown in vitro. Adenoviruses that were unable to grow in vitro were also identified by Richmond et al. (1979) in children during an outbreak of diarrhoea at an R.A.F. base in England. Brandt estimated that adenoviruses of this kind could account for 3.6% of the diarrhoeas caused in children in Washington D.C. (Brandt et al. 1979).

We have performed studies of the genetic variability of adenoviruses since

1965 and were intrigued by the fact that the majority of the adenoviruses associated with gastroenteritis could not be grown *in vitro*. This feature is a characteristic of most viruses which have been causally associated with gastroenteritis. This property also distinguishes the fastidious enteric adenoviruses from all other known adenoviruses. Their inability to grow *in vitro* hampers conventional identification and typing in neutralization assays. We have therefore analyzed the virion polypeptides of all established adenovirus serotypes by SDS-polyacrylamide gel electrophoresis. This technique could be used to divide the established adenovirus types into five subgroups (Wadell, 1979). The fastidious enteric adenoviruses displayed a polypeptide pattern, which was so distinct that they should be classified in a separate sixth subgroup of adenoviruses (Wadell et al. 1980). This conclusion was supported by an elaborate analysis of the genomes of all adenovirus prototypes and the fastidious enteric adenoviruses by DNA restriction endonucleases. This study yielded a system for classification of adenoviruses which is based on the characteristic number and sizes of the DNA restriction fragments. It was also confirmed that the fastidious enteric adenoviruses form a sixth subgroup of human adenoviruses (Wadell et al. 1980). It was our aim to prepare specific sera for direct detection of fastidious

enteric adenoviruses in order to determine their distribution and pathogenic relevance. We were faced with the problem to prepare clean immunogens from stools. A new immunization method "affinity bead immunization - ABI" (Johansson et al. 1979) was therefore developed. In this procedure antibodies specific for adenoviruses are covalently linked to the sepharose beads. Stools containing enteric adenoviruses are bound to the solid phase and contaminating material can then easily be rinsed off. Adenoviruses antigens can, however, not be eluted from the antibody solid phase with preserved immunogenicity. The antigens bound to the solid phase - the bead - were therefore used as immunogens. The bead serves in this case as a carrier and a specific antibody response can be obtained with only 50 nanogram antigen. It is of particular importance to use specific reagents prepared by affinity chromatography since rabbits frequently carry antibodies against human rota viruses (Jacobsson et al. 1979).

The reagents specific for fastidious enteric adenoviruses were evaluated in an immune electro osmophoresis technique (Jacobsson et al. 1979) and also in Elisa assays (Johansson et al. 1980). The Elisa technique is the most versatile technique. The first Elisa assay is groupspecific and provides a means for direct identification of all adenovirus

types. This test is thus a complement or an alternative to identification by the electron microscope. The second assay is type specific and identifies fastidious enteric adenoviruses the (candidate adenovirus type 38). We have identified this fastidious enteric adenovirus in specimens from Helsinki, Stockholm, Oslo, Glasgow, Bristol, Birmingham, Sorento, Washington D.C. and Dacca.

3. Rationale:

There are several reasons to determine the etiology of diarrhoeas in greater detail.

1. to determine those which are most common and cause the most severe disease and sequalae.
2. to look for specific modes of transmission with an aim to future prevention- to examine the immune response and its effectiveness in preventing subsequent disease.
3. to identify virus strains which can serve as candidates for vaccine preparation.
4. to develop methods to diagnose the illness caused by an agent and to evaluate the response to specific treatment.

Over the past decade, our ability to establish a diagnosis in a patient with diarrhoea has improved greatly as simple

diagnostic test for ETEC, Rotavirus, Campylobacter have become available. We can now identify agents associated with 83% of pateints with diarrhoea attending the Dacca hospital. The proposed study will let us know how important FEAs' are among the remaining group of patients whose diarrhoea is currently without known etiology and the relative prevalence of this agent in comparison to a community control group of individuals of the same age. If FEAs' are found to be important in Dacca, further work will be directed to setting up appropriate diagnostic tests in Dacca pursuing more indepth studies of the epidemiological, clinical and immunological aspect of the disease. We will also be able to correlate FEAs' with the presenting symptoms found in the hospital surveillance system.

4. Methods:

Epidemiology and clinical features:

The study will begin with a six - twelve months pilot phase. Stool samples from 3 groups will be collected and analyzed for adenovirus:

Group A: A stool specimen from every 3rd patient registered in the Dacca hospital surveillance system will be collected for analysis. Currently, a 4% sample of the 100,000 patients

seen at the Dacca hospital each year (N=330/month) are examined for diarrhoeal disease symptoms, etiology, stool microscopy and demographic information (expected sample size - every third patient x 330/month x 6 month = 660).

Group B: From these patients, no etiological agent can be found in 18%. All specimens from this group will be tested for PEAs'. (Expected sample size - 18% x 330 pts/m. x 6 Month = 356).

Group C: A community control group comprised of healthy family contacts of patients seen at the Dacca hospital for diarrhoea due to an agent other than adenovirus. Stool specimens form these patients are routinely being collected in family studies of Campylobacter and cholera patients and will be examined for adenovirus as well.

Logistics: Stool specimens collected in Dacca will be frozen in a 20% suspension with PBS and handcarried to Sweden in the middle and the end of phase 1. Upon receiving these results, we will consider further studies of family contacts, looking for modes of transmission and the development of an immune response.

Laboratory Aspects - phase 1

The stool specimens will be analyzed in Sweden by Elisa

techniques specific for all adenoviruses and for specific adenoviruses.

Electron microscopy and the highly sensitive immune electron microscopy method according to Nicolaieff et al. (1980) will also be applied. In addition techniques of molecular virology will be used to determine the genome types of adenoviruses. The genome types of adenoviruses are determined by the use of DNA restriction endonucleases (Wadell et al. 1980).

It is valuable to be able to perform the adenovirus group specific Elisa assay and the Elisa assay specific for fastidious enteric adenovirus in connection with analysis of the adenoviruses isolated. This approach has already allowed the identification of additional serotypes of fastidious enteric adenoviruses. Specific sera against these recognized sero types are being prepared.

Information on the genetic variability within the serotype designation can be of substantial value when isolates obtained from controls and patients with symptoms are compared. There is a possibility to identify virulent and less virulent genome types. We have previously exploited this possibility to large extent in studies of

adenovirus type 7 which is the most frequently identified cause of respiratory outbreaks among human adenoviruses. In this virus type we have analysed the genomes of 176 strains from Europe, USA, Africa and Asia and identified 4 different genome types. Two of these are highly virulent and account for 95% of the isolates from patients, whereas two are less virulent and are isolated from healthy shedders (Wadell et al. submitted for publication). The adenovirus genome appears to be very stable indeed since there is no difference between the American vaccine strains against adenoviruses type 7 after 35 passages in vitro and the 5 different wild type strains which we have found. The American vaccine strain provides efficient protection against respiratory outbreaks among military recruits.

It is possible that this approach could be used also in the attempts to create immunoprophylaxis against the viruses causing diarrhoeas. Live virus vaccines should be expected to induce efficient gut immunity.

Phase 2:

If FEAs' are found to be an important agent of diarrhoea from phase 1 studies, further work will be directed to

bringing the specific Elisa assay and reagents to Dacca to conduct further epidemiologic family studies.

D. SIGNIFICANCE

This study will help us further our understanding of the currently undiagnosed causes of diarrhoea. If FEAs' are found to be important by their relative high prevalence or by the severity of disease with which they are associated this study will provide the impetus to bring the Elisa test to Dacca to initiate further clinical, epidemiologic and laboratory based studies and will promote interest in further investigations concerning the possibilities for vaccines with FEAs'.

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SECTION III - BUDGET

A. DETAILED BUDGET

Phase 1: All laboratory work will be done in Sweden. Additional expenses in Dacca limited to collecting data and specimens, preparing, freezing, packing specimens, and analyzing results. At the end of phase 1 the collaboration will be reviewed to determine the importance of FEA's and the utility of setting up the diagnostic test in Dacca as part of more extensive studies.

	1. Additional Expenses:	% of Time	Taka	Dollar
	Dr Roger Glass	5%		1000
	Dr M.I. Huq))	Time included in surveillance protocol		
	Dr B.J. Stoll)			
	Lab. Assistant	25%	5,000	
	Field Workers - 2	25%	10,000	
2.	SUPPLIES AND MATERIALS			
	Storage vials - 1000			50
3.	EQUIPMENTS:			
	Deep Freeze space - Nil			
4,5.	PATIENT HOSPITALIZATION:			
	Outpatient care - Nil			
6.	ICDDR,B TRANSPORT: 4 Tk./mile (2000 miles) 8000			
7.	TOTAL TRANSPORTABLE PERSONS: Nil			
8.	TRANSPORT OF SPECIMENS (excess baggage):			200
9.	RENT, COMMUNITY UTILITIES: - Nil			

		Taka		Dollar
10.	Printing, Reproduction: -	2,000		
11.	Other Services : - Nil			
12.	Construction, Renovations: - Nil			
	Summary -	Tk. 25,000	US$	1,250

US$ 2,500.00

CONSENT FORM

To be read to patients without diarrhoeal disease as controls for adenovirus study.

Doctor's at the Cholera Hospital are concerned about the role of a specific virus in causing diarrhoea. While you do not have diarrhoea, we would like to examine a stool specimen from you (or your child) and see if this virus occurs in patients without diarrhoea as well. If you would bring a stool specimen for this study, we will also examine it for other worms or ameba and can provide treatment where appropriate and without cost. You do not have to participate in this study and any information collected will not mention you by name or identify you in any way.

Thank you for your help.

81-053

সম্মতি পত্র

অ্যাডেনোভাইরাস গবেষণার তুলনার জন্য যে সমস্ত রোগীদের পাতলা পায়খানা বাই তাদের পড়ে শুনানোর জন্য।

কলেরা হাসপাতালের ডাক্তারগণ পাতলা পায়খানার জন্য দায়ী এক বিশেষ ভাইরাস জীবাণু সম্বন্ধে গবেষণা করছেন। যখন আপনার পাতলা পায়খানা থাকবে না তখন আমরা আপনার (অথবা আপনার বাচ্চার) পায়খানা পরীক্ষা করে দেখতে চাই যে পাতলা পায়খানা ছাড়াও এই ভাইরাস জীবাণু শরীরে থাকে কি না। আপনি পায়খানার কিছু অংশ গবেষণার জন্য দিলে তার মধ্যে আমাশয় বা গ্রিশমি জাতীয় কোন জীবাণু আছে কি না তাও পরীক্ষা করে দেখা হবে এবং বিনা খরচে উপযুক্ত চিকিৎসা করা হবে। এই গবেষণায় আপনার সম্পূর্ণ অংশগ্রহনের প্রয়োজন নেই এবং আপনার সম্বন্ধে সংগৃহীত তথ্যে কোথাও আপনার নামের উল্লেখ থাকবে না।

আপনার সহযোগিতার জন্য ধন্যবাদ।