COLLABORATIVE RESEARCH: BETWEEN ICDDR,B/IPH WITH EXTERNAL RESOURCES/FUND

Date 26 Nov 84

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator: Dr. T.C. Butler and Dr. Farida Huq (IPH) Trainee Investigator (if any)

Application No. 84-046

Supporting Agency (if Non-ICDDR,B)

Title of Study: Causes of Acute Lower Respiratory Tract Infection in Children of Bangladesh

Project status:
(X) 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:
   ___ 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.

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

NOV 26 1984

Trainee

NATIONAL ACADEMY OF SCIENCES
BOARD OF SCIENCE AND TECHNOLOGY FOR INTERNATIONAL DEVELOPMENT
RESEARCH GRANTS PROGRAM

International Centre for Diarrhoeal Disease Research, Bangladesh
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Causes of Acute Lower Respiratory Tract Infection in Children of Bangladesh

Acute Respiratory Infections in Children

Research Project Director

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ABSTRACT

Acute lower respiratory tract infection is a leading cause of morbidity and mortality in children of Bangladesh. Bacterial causes are believed to be important but viral agents have not been defined. At our hospital for diarrhoeal diseases, pneumonia is a common cause of admission, and about 10% of these admitted patients die. The present study proposes to identify, by culture methods, viral and bacterial agents in 500 of these patients and in 500 cases of acute lower respiratory infection at Dhaka Medical College. The first phase will be to upgrade virology by strengthening the present virological lab at the Institute of Public Health and by recruiting and training personnel during the first 6 months. The second phase will be to select 1000 children of ages 0-4 years who show signs and symptoms of acute lower respiratory tract infection for complete viral and bacterial culturing. This work will cover a 2-year period. Other phases will include post-mortem examinations of fatal cases, bacterial carriage in non-ARI controls, chlamydial isolation, and data analysis. The final 6 monthswill be used to analyze data, make recommendations for treatment, and plan a follow-up study in a rural community in Bangladesh. It is expected that these results can be used to recommend rational use of antibiotics selectively to decrease fatalities from respiratory tract infection.

BACKGROUND

In Bangladesh, as in other developing countries, childhood mortality rates remain higher than in developed countries. The two leading causes of death in children in developing countries are infectious diarrhoea and respiratory infection. Bacterial and viral pathogens are the causative agents in the majority of cases of both diarrhoeal and respiratory illnesses, but the specific agents causing each kind of disease are usually different. Although physicians in developing countries recognize that respiratory infections are common and serious, too little is known about their causes and epidemiology to formulate a rational approach to treatment or to prevention.

Bulla and Hitze of the WHO in Geneva wrote a review in 1978 which gave a good perspective on Acute Respiratory Infections or ARI (Bull. WHO 56(3): 481-498, 1978). Using data from countries reporting information on ARI to the WHO, they determined that in Asian countries in 1970-1973, there were 128,000 deaths from ARI in a population of about 227 million. The deaths due to tuberculosis were 50,000 and to chronic lung disease 26,000. Thus, ARI were responsible for 62.5% of the deaths from respiratory disease. Considering deaths from all causes in all reporting countries, deaths due to ARI accounted for 6.3% of the deaths.

Mortality rates due to ARI were shown by Bulla and Hitze to be highest in infancy and to decline in older children stepwise in children 1-4 and further in children 5-14 years old. In infants of Asian countries,

the mortality rate from ARI was 1,242 per 100,000; in children 1-4 it was 204 per 100,000; and in children 5-14, it was 23 per 100,000.

The epidemiology of ARI in childhood is a changing scene with age in which viral and bacterial agents play various roles that are determined by immunological status and exposure to the causative agents. In the first few months of life, maternal antibodies may provide some protection although some antibodies, such as, against respiratory syncitial (RS) virus, may form sensitizing complexes with the virus. With increasing age, exposure to various infective agents and/or cross-reacting antigens allows the child to acquire immunity to some of the respiratory pathogens. Some viruses and bacteria have seasonal patterns of occurrence. Antigenic variations of influenza viruses permit epidemics to move through populations that have immunity against prior viral strains.

From the rather lengthy lists of pathogenic agents for ARI, their relative importance in developing countries has not been established. Most of them are present in developing countries with relative prevalances that are similar to developed countries. These include bacterial infections due to pneumococci, Klebsiella pneumonia, streptococci, staphylococci, Neisseriae, Hemophilus influenzae, Pseudomonas, and Enterobacteriaceae. Other bacteria that could be causes of pneumonia in developing countries are Bordetella pertussis, and Legionella pneumophila. Viruses include adenoviruses, influenza and parainfluenza, RS virus and rhinovirus. The chlamydial agents are present, as well as, Mycoplasma and Coxsiella burnetti. Bacterial superinfections of the respiratory

tract following upper respiratory infections or other systemic disease need to be recognized. Pneumocystis carinii is an important protozoal cause of pneumonia in immunosuppressed patients.

The importance of viruses as causes of ARI in Asian developing countries has been established in India. A survey in villages of West Bengal by Kloene et al (Am J Epidem, 92 : 307, 1970) showed that respiratory viruses were frequently isolated. These included influenza, parainfluenza, adenovirus, rhinovirus, and RS virus. A study in Vellore by Steinhoff and John (Workshop on ARI, Univ North Carolina, May 1983, p.1032) revealed that children with ARI had viruses isolated from 32 of 184 cases; the most frequently recovered virus was parainfluenza 3, followed by influenza B and RS virus.

There is a controversy over the question whether viral agents or bacterial agents predominate as causes of lower respiratory infection in children of developing countries. It is essential to resolve this issue because of the therapeutic implications. If viruses prove to be ascendant, the current practice of using antibiotics to treat ARI may be useless or even harmful to infected patients.

In developing countries, the persistence of measles in both endemic and epidemic forms is also an important feature of childhood disease. Measles is often virulent and may cause death, sometimes from respiratory tract superinfection. Another factor of child health in developing countries is poor nutritional status. Poorly nourished children who get infected are

prone to have more serious diseases, due to either poor metabolic reserve or to immunodeficiency associated with malnutrition.

SCIENTIFIC ANTECEDENTS

Since 1978, when the Cholera Research Lab was changed into the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), much effort has been devoted to reducing diarrhoeal mortality by promoting the use of oral rehydration solution (ORS). In some areas of the country, the widespread acceptance of ORS has produced signs of reducing mortality rates due to diarrhoea. Correspondingly, we may expect to see a higher percentage of childhood mortality due to ARI in Bangladesh.

During the recent years of 1982, scientists at the ICDDR,B have recognized the need to understand ARI more fully and have carried out 3 relevant studies. The preliminary information from these 3 studies lays the groundwork for this research proposal and will be summarized here. These studies include a new postmortem study, a diagnostic study of pneumonia, and a serosurvey for chlamydia infection.

To establish the causes of morbidity and mortality in certain diarrhoeal diseases in Bangladesh, 50 deceased diarrhoeal patients with dysentery or other signs of invasive intestinal disease were subjected to postmortem examination. Antemortem clinical data, as well as, postmortem laboratory and pathological data were assembled in order to determine causes of death. The most common diarrhoeal pathogens identified were Shigella species in 13 cases, Entamoeba histolytica in 8 cases, and Campylobacter jejuni in 6 cases. The most frequently identified anatomic

diseases were fatty-degeneration of the liver in 32 cases, bronchopneumonia in 24 cases, Shigella colitis in 13 cases, necrotizing pneumonia in 12 cases, acute bronchitis in 9 cases, and amebic colitis in 7 cases. The causes of death, both immediate and contributing causes, were judged to be marasmus or kwashiorkor in 23 cases, bronchopneumonia in 19 cases, septicemia in 16 cases, Shigella colitis in 13 cases, necrotizing pneumonia in 10 cases, amebic colities in 7 cases, hypoglycemia in 6 cases, hemorrhagic necrosis of the intestine in 5 cases, and hypokalemia in 5 cases. All patients had between 3 and 10 diseases established after the postmortem examination was completed. These findings showed that patients dying with invasive diarrhoeal diseases in Bangladesh frequently have advanced severe diseases affecting the intestine, liver, lung and other organs. From the few cases, pathogenic bacteria, including pneumococci, staphylococci, and Gr -negative bacilli, were isolated; but many cases had no cause identified.

Postmortem examinations are rarely carried out in developing countries because of attitudes of people and lack of facilities and trained personnel. Thus, the postmortem capability at ICDDR,B is a valuable resource for learning more fully about causes of death in developing countries.

In a diagnostic survey of respiratory infection in Bangladesh, 70 patients with positive chest x-ray were studied. Out of 70 cases involved in the study, 10 children died in hospital. Another died at home having left the hospital giving risk bond. Pneumococcus was the most common organism isolated alone or in combination with Gram-negative organisms

including Haemophilus, E. coli, Klebsiella and Pseudomenous. Mycobacterium was isolated in three cases. Bordetella pertussis was isolated in 2 cases. Antigen for pneumococci was demonstrated by CIE in 33/48 of confirmed cases of pneumonia. The technique was easily performed in ICDDR,B and gave rapid results.

In a survey for antichlamydial antibodies, which was the first attempt to detect evidence for chlamydial infection in Bangladesh, sera of 93 patients, who were part of a 4% systematic sample of patients visiting a hospital for diarrhoeal diseases from April-June 1982, were examined. Sera were tested for both IgM and IgG classes of antibodies using the smplified microimmunoflourescence assay described by Wang et al. Sera reacting only with serotypes A, CJ, or I were considered negative because of possible lack of specificity of these antigens. Stool samples were obtained for detection of diarrhoeal agents including Salmonella species, Shigella species. Vibrio cholerae, Campylobacter jejuni, enterotoxigenic Escherichia coli (ETEC) rotavirus, Entamoeba histolytica and Giardia lamblia and for enumeration of leukocytes and red blood cells. Antibody titers $\geq$ 1:8 were considered positive and were observed in 25 patients. Four patients showed elevated IgM antibody and 21 patients had elevations of only IgG antibody. Convalescent sera were obtained from 7 patients 2 months after the initial serum collections, and 2 of these patients showed a fourfold change in IgM antibody titer. The 25 seropositive specimens reacted with a variety of the 9 antigen pools tested, and no single antigen was positive in all 25 cases.

The high prevalence of antichlamydial antibodies (25/93) in this sample of diarrhoea patients suggests that chlamydial infections are common in Bangladesh. Bangladesh is not an endemic area for trachoma. Sexually transmitted infection, passively acquired antibody in young infants, and perinatally acquired infection may account for the observed seropositivity. This is the first report of chlamydial serology in this country, and seropositivity rates in patients without diarrhoeal diseases are not known. Although none of these patients had pneumonia, chlamydiae are an important cause of neonatal pneumonia and should be looked for in developing countries.

COLLABORATION BETWEEN ICDDR,B AND INSTITUTE OF PUBLIC HEALTH (IPH)

The ICDDR,B and IPH are located in the same building in Dhaka and share some of the support facilities for the laboratories. Dr. Farida Huq is the Head of Microbiology and in charge of viral diagnosis. The present laboratory carries out virus isolation in embryonated eggs and does serologic testing by complement fixation. The lab also has tissue culture capability and is maintaining 4 cell lines: Vero cells for measles virus, BHK for rabies and rubella, He La cells for polio virus; and HEP-2 cells for polio virus. Upgrading of this laboratory will be necessary to add the human kidney cell line for respiratory viruses. The ICDDR,B has 2 fluorescent microscopes that can be used for confirmation of virus identification. The ICDDR,B has deep-freezers, centrifuges, and a laminar flow-hood which can be shared with IPH during the upgrading process so as to avoid purchase of excessive amounts of equipment needed for virology upgrading.

OBJECTIVES

1. General

   A. To determine the prominent infectious causes of lower respiratory tract infection in hospitalized children of Bangladesh so that rational approaches to treatment and prevention can be formulated.

   B. Make correlations between etiologic agents and the clinical and pathological features of lower respiratory tract infection in hospitalized children of Bangladesh so that rational approaches to treatment and prevention can be formulated.

2. Specific Objectives

   A. To establish a modern, efficient Virology Laboratory for isolation and identification of respiratory viruses by tissue culture technique.

   B. To identify common viral and bacterial causes of lower respiratory tract infection in 1000 hospitalized children. These patients will include 500 patients at Dhaka Medical College without diarrhoea and 500 patients at ICDDR,B with diarrhoea.

   C. To carry out postmortem examinations in fatal cases of respiratory tract infection in children of Bangladesh.

   D. To describe clinical and pathological features of lower respiratory tract infection in children of Bangladesh and make correlations with identified etiological agents.

E. To assess prevalence of chlamydial pneumonia by using McCoy cell culture method.

F. To determine carriage rates of bacterial pathoqens in children without ARI.

RESEARCH PLAN

PHASE - I: Virology Laboratory Strengthening

A. Hypothesis

Children of Bangladesh with lower respiratory tract infections will have respiratory viruses, including influenza virus, para-influenza virus, respiratory syncitial virus (RSV), adenovirus and rhinoviruses. These viruses can be cultured using tissue culture techniques in our Virology Lab after it is strengthened.

B. Methodologies

1. Collection of Specimen: A wire nasopharyngel swab is passed into the nostril parallel with the palate and qently rotated. The secretions are eluted from the swab in the transport medium containing 3 ml of tryptosephosphate broth (pH 7.0-7.2) containinq 0.5% gelatin. Specimens are transported to the laboratory on ice and maintained at 4°C before inoculatinq. Freezinq of the specimens will be avoided. Antibiotics will be added before inoculation to yield a final concentration of 800 units of penicillin and400 microgr of streptomycin per ml. Before inoculation, the specimens are centrifuged at 4°C for 15 minutes at 1500 X g.

Influenza Viral Isolation

A. The isolation will be done in primary monkey kidney (PRMK) cells. The cells will be washed twice with Hanks BSS and will be maintained in serum free medium. The tubes will be incubated in roller drum at 35°C. After appearance of CPE, HAD test with chicken and guineapig RBC will be done. The HAD test will be done routinely at 5 and 10 days after inoculation. If the presence of HAD is observed, then HA test will be done. If HI titre is found to be low, then second passage will be done in a fresh set of tubes. Confirmation will be done with HAI, NT or type specific CFT or DID. Subcultures will be done after 7-10 days.

B. Egg Inoculation - 10-11 days incubated eggs will be used. Allantoic and amniotic cavity inoculation will be done. Eggs will be kept at 33-34°C for 2-3 days. The fluids collected from Allantoic cavity will be subjected to HA test with chicken RBC to detect presence of virus. The confirmation of the virus will be done as the same as in tissue culture.

2. Isolation of Parainfluenza

Samples will be collected in the same manner as that for influenza. Isolation will be done similar to influenza virus in RPMK at 35°C. Identification will be made by HAD with guineapiq RBC. Confirmatory tests will be done for different types by HAI test using specific antiserum.

3. Isolation of RSV

Collection of specimen is the same as that for influenza virus. For inoculation, HEP-2 or Hela cell lines maintained in Eagle basal medium plus an equal volume of medium 199 with 2% fetal calf serum will be used. Cultures will be incubated at 36°C after the appearance of CPE, identification of virus will be done by infectivity neutralization and by FA staining. Infectivity neutralization will be done with known antisera which is mixed with the cultured virus and planted in a new cell line. Positive result will be obtained where no CPE will be seen. FA staining will be done by fluorescent labelled antisera and observing with fluorescent microscope to see the stain taken up by the infected cells.

4. Isolation of Adenovirus

Collection of specimens will be the same as that for influenza virus. The specimen will be inoculated in the HEP-2 cell line. The inoculated cultures will be incubated at 36°C. After the development of characteristic CPE, the tests for the identification of virus will be done. These will be FA test and HAI test. Viral isolates will be frozen and stored for future typing in the event of an epidemic of adenovirus infection.

5. Isolation of Rhinovirus

Because rhinoviruses are likely to be less important than the other viruses in causing lower respiratory tract disease, detection of

this virus will be deferred until the second year of work. A reduced sample of 200 children from the study will be examined. The naso-pharyngeal specimen will be placed into veal infusion broth with 1% bovine serum albumin. The cell line used will be Wl-38. The lines will be incubated at 33°C at a pH between 7.2-7.4. After the observation of characteristic CPE, the following tests will be done to prove the rhinovirus infection:

a. Stability on exposure to lipid solvents such as chloroform or ether.

b. Complete or almost complete inactivation on treatment at pH3 distinguishes rhinovirus from Enterovirus, the latter being acid resistant.

C. Inputs Required

1. The first step is the strengthening of Existing Viral Laboratory for support of the investigations.

The following are needed for strengthening:

A. A tissue culture lab with laminar flow, UV light, and airconditioner.

B. A viral inoculating room which may be gotten by converting the existing room into small cubicles fitted with UV light, laminar flow, and airconditioner.

C. A serology room which may be gotten by converting the other existing room into small cubicles fitted with UV light, laminar flow, and airconditioner.

D. New instruments are needed:

1. Laminar flow. One is needed to add to the one in the lab now.
2. Sensitive balance
3. pH meter
4. Millipore filtering units with membrane of different porosity
5. UV light

E. Laboratory supplies, including:

1. Tissue culture meida
2. Various antisera
3. Chemicals
4. Plastic tissue culture bottles
5. Tissue culture plates, diluter

2. Personnel

A. Dr. Farida Huq will be in charge of the Virology Lab

B. One Research Officer with Masters Degree, full time

C. Two Technicians, full time

D. One Laboratory Assistant, full time

E. Visiting Virology Consultants. Two will come for 1 month each during the first 2 years

3. Training

A. Dr. Huq will go to UK or USA for 2 months for refresher training in the same laboratory of the above designated consultant.

B. Dr. Huq will give on-site training to the Research Officer and Technicians.

D. Timetable

The first 6 months will be used for obtaining new equipment, reorganizing the lab spaces, training for Dr. Huq abroad, and on-site training of other personnel. After the start-up phase, the lab will be ready to receive clinical specimens. One Visiting Consultant will be scheduled to come at the end of this 6-month period and the second Consultant a year later.

PHASE 2 : Clinical Studies

A. Hypothesis - Children in Bangladesh with acute lower respiratory tract infection will harbor pathogenic viruses and bacteria in their respiratory tracts that have diagnostic value. These etiologic agents can be isolated and identified in our laboratory. A hospital-based population under study will give valuable information for future community studies.

B. Methodologies :

1. Patient selection. Five hundred patients 0-4 years old coming to the ICDDR,B hospital with histories suggestive of acute lower respiratory tract infection will be considered. All patients coming to ICDDR,B have diarrhoea but many have insignificant amounts of diarrhoea with major respiratory complaints, and mostly these will be selected. An additional 500 children at Dhaka Medical College 0-4 years old with histories suggestive of acute lower respiratory tract infection and without diarrhea will be recruited. To be

selected patients must have the onset of respiratory symptoms within 7 days, have not been treated with antibiotics and have at least 3 of the following features:

i. cough productive of purulent sputum

ii. flaring of alae nasae or intercostal retractions

iii. respiratory rate $\geq$ 40 per minute

iv. temperature $\geq 39^{\circ}C$

v. rales on anscultation or dullness to percussion

To ensure a steady flow of patients throughout the 2-year study period, approximately 5 patients per week will be selected from each hospital. To reduce selection biases, the study team will visit each hospital regularly 3 days a week and screen eligible patients. If more than the required number (approx. 3 patients) meet the selection criteria during a visit, the team will select 3 patients randomly using a table of random numbers and the last 2 digits of the hospital identification numbers.

Selected patients will have a history obtained for risk factors and have a physical examination and x-ray. The weight and height after initial rehydration will be measured.

2. Sample size

1000 children will be entered into the study

3. Controls

To determine the carriage rates of pathogenic bacteria, 100 control cases without ARI at ICDDR,B and 100 control cases without ARI at Dhaka Medical College will be selected for throat swab culture. The controls

will be matched by age group 0-1 yr or 1-4 yr with ARI cases that were selected at the same time. They will be recently hospitalized and not receiving antibiotics at the time of selection.

4. Bacteriological studies

A. Specimen collection - Specimens for virology will be collected as described on page 10. Specimens for bacteriological examination will be collected at the same time as those for viral examination. A cotton swab on a stick will be used to obtain secretions from the oropharynx.

Test to be performed on these swabs.

B. The specimen after standard processing methods will be streaked onto

1. blood agar plate
2. supplemented chocolate agar plate
3. MacConkey agar plate
4. Bordet-Gengou medium for Bordetella
5. mycoplasma media
6. CYE agar Legionella

The plates will be examined using standard bacteriologic procedures. The organisms to be considered are:

Streptococcus pneumoniae

Haemophilus influenzae

Group A streptococci

Bordetella pertussis

S. aureus

K. pneumoniae

Escherichia coli (and other enteric bacilli)

Proteus

Pseudomonas aeruginosa

L. pneumophilia

Confirmation tests will be done.

Str. pneumoniae - optochin

serotyping

H. Influenza - X - v discs

H. infl-antiserum type B by slide agglutination

Group A

Streptococci - Bacitracin

Serogrouping

S. aureus - Coagulase

Mannitol

A blood culture will be obtained for all patients

A chest x-ray will be taken and interpreted. Patients will be selected regardless of whether pulmonary infiltration is present.

C. Inputs required:

1. One research officer with Masters degree in Microbiology
2. Dr. Nigar Shahid - physician - 50% of time
3. One technician - full time
4. One laboratory assistant, full time
5. Visiting Microbiology Consultant - 30 days in first year
6. All required equipment is on hand in the ICDDR,B Microbiology

D. Clinical Courses

The selected patients will all be hospitalized and followed for outcome. Those with culture results indicating bacterial infection will be treated with appropriate antibiotics.

E. Timetable

Two years will be required.

PHASE 3: Post-mortem Examinations

A. Hypothesis

Children who die with clinical or radiographic evidence of respiratory infection will have certain histopathological features that have diagnostic importance. Special stains of lung tissue will reveal etiologic agents that could not be identified by routine ante-mortem testing.

B. Methodologies

The 1000 cases of acute lower respiratory infection selected for clinical study will be followed. Those who die in the hospital will be selected for post-mortem examination and their families approached for permission. The pathologist will examine the lungs for gross evidence of pneumonia. The lungs will be filled with formalin solution by instillation through the trachea in order to inflate collapsed areas. Lungs will be cut and slides of touch impressions made for Gram-stains and acid-fast stains. Tissue blocks of affected areas will be made for staining with hematoxylin-eosin, Brown-Brenn stain, PAS, Ziehl-Nielsen, and methenamine silver stains. This will allow

description of histologic type of pneumonia by the International Classification of Diseases according to the WHO, including broncho-pneumonia, interstitial pneumonia, and necrotizing pneumonia. Special stains will show bacteria, mycobacteria, fungi, and protozoa (Pneumocystis).

C. Inputs Required:

1. Pathologist Consultant - 10% of time
2. Histology Technician - 50% of time
3. Pediatric Pathologist - Visiting Consultant for 1 month after one year of work accomplished.
4. New equipment :
   a. Tissue processor
   b. Microtome

D. Timetable

This phase will take 2 years and coincide with the clinical studies.

PHASE 4 : Chlamydial Isolation

A. Hypothesis

Some children with acute lower respiratory tract infection will be shown to have chlamydial infection of the respiratory tract.

B. Methodology

Patients will be selected who are 0-1 yr old in order to get the highest risk children for this disease. The method of culture in

McCoy cells as recommended by WHO will be used. Coverslips are placed on the bottom of flat-bottom tubes. Cell maintenance medium with antibiotics will be prepared containing Wellcome Minimal essential medium, fetal calf serum, vancomycin and bicarbonate.

McCoy cells will be periodically passed and stored in this medium. McCoy cells are transferred to the tubes to form monolayers. Bronchial secretions and minced lung tissue will be placed in antibiotic-containing borth and emulsified by shaking with glass beads. The inoculum is then placed into the tubes containing coverslops with McCoy cells. The tubes are centrifuged at 3000 X g for 1 hr. The medium is removed and replaced with growth medium containing cycloheximide. The tubes are incubated for 65 hrs. at 37°C. Coverslips are removed, stained with Giemsa stain, and examined for inclusions under dark field microscopy.

C. Inputs Required

Dr. Bennish has experience with the culture technique that he gained with Dr. Beem in Chicago and Dr. Schachter in San Francisco.

D. Timetable

The chlamydial isolations will be attempted continuously during the 2 years of the clinical studies.

PHASE 5 : Analysis of data and recommendations

A. Methods

By use of the ICDDR,B computer facility and by hand calculators, the data will be analyzed initially after the first year of the project. Deficiencies in the design and data collection will be corrected at this time. All data will be analyzed intensively during the final 6 months. No new cases will be examined during this time. Conferences will be held among the participating physicians, pathologist and microbiologists.

All the data will be considered together and correlations made between etiologic agents and the pathological and clinical features. One or more research papers will be written. Recommendations for antibiotic treatment will be formulated. Extension of this study to the Matlab field area will be considered as a future study to gain more accurate epidemiological information for the community.

B. Inputs required

The statistical staff of the ICDDR,B will be consulted. Computer time will be reserved for data read-out and for statistical tests. Half-time of a secretary will be used to type the results and manuscript.

C. Timetable

This work will be carried out in the final 6 months of the project. Dr. Butler will have returned to the USA by this time, and he will go to Dhaka once to assist with the final stages of data analysis.

Resources

The ICDDR,B was formerly the Cholera Research Laboratory and now is an international centre that is in Bangladesh. It receives financial support from more than 30 countries and agencies. It is governed by a Board of Trustees, whose members are mostly from developing countries. The Minister of Health of Banqladesh is a Trustee Member. It has linkages with the research organizations in Bagnladesh and with collaborating institutions outside the ocuntry. The ICDDR,B runs a hospital that treats about 80,000 patients a year in Dhaka and has field facilities in other areas of the country. The ICDDR,B employes about 1,000 persons and has a fleet of vehicles. It has a good medical library, a computer, animal house, microbiology lab., histopathology lab., clinical pathology lab., and morque.

The Institute of Public Health (IPH) is housed in the same building as the ICDDR,B. The IPH has a laboratories for the confirmation of communicable diseases in Bangladesh. The Virology Lab. is located in IPH and receives support form the Government of Bangladesh. Dr. Huq's salary is paid entirely by IPH. The Dhaka Medical College Hospital is the major teaching hospital in Dhaka supported by the Government. It receives indigent patients and Dr. Naimun Nahar is Associate Professor of Pediatrics. She will not receive salary from this grant and will assist in the selection of patients.

The curriculam vitae of the Investigators are attached.

Follow up

The research results will be presented at scientific meetings and published in sicentific journals. Specific recommendations based on the findings will be made to public health officials regarding epidemic potential for tuberculosis or influenza. Long-term recommendations will be made as deemed appropriate by the results.

DETAILED BUDGET

(U.S. DOLLARS)
1985

Salaries

Staff

	Name	Title	Annual Salary	% of Time devoted to work	Salary charged to project
1.	Dr. F. Huq	Virologist	5,000	50%	-
2.	Dr. N. Shahid	Physician	4,000	50%	2,000
3.	Dr. T. Butler	Coordinator	67,000	20%	-
4.	Dr. N. Nahar	Padiatrician	4,000	10%	-
5.	Dr. M. Islam	Pathologist	30,000	10%	3,000
6.	Dr. M. Bennish	Chlamydiologist	40,000	10%	-
7.	To be named	Virology Res.Offi	3,000	100%	3,000
8.	To be named	Microb. Res.Offi	3,000	100%	3,000
9.	To be named	Technicians (3)	7,500	100%	7,500
10.	To be named	Lab. Assistant	5,000	100%	5,000

(Fringe benefits included in salaries)

Consultants International

	Name	Function	No.of days	Proposed Daily rate	Total cost
1.	To be named	Virologist	30	70	2,100
2.	To be named	Microbiologist	30	70	2,100
3.	Dr. F. Huq	Microbiologist for training	45	60 (according to Bostid rules)	2,700

Travel International:

	Name/Title	Number of trip	Purpose	Cost per trip	Total cost
1.	Virologist	1	Consultancy	2,500	2,500
2.	Microbiologist	1	Consultancy	2,500	2,500
3.	Dr. F. Huq	1	Training	2,500	2,500

Materials, Services, and Training

Purpose	Location	Cost
Virology supplies	Dhaka	10,000
Microbiology supplies	Dhaka	5,000
Histology supplies	Dhaka	2,000
Radiology supplies	Dhaka	1,500

.../2

Detailed Budget - 1985 (Continued)

Equipment purchased internationally

	Items	Cost
(Purchase price plus shipment 10%)		
	1 Laminar flow hood, Baker, 4ft, floor model	5,600
	1 Incubator, water jacketed, floor model	5,300
	1 Modular rotator to fit into incubator	1,380
	1 Mettler balance	4,130
	1 Millipore filtration unit with vacuum pump	440
	3 UV lights	500
	1 pH meter, corning	880
	Tissue processor, Tissue Tek II (Lab-Tek 4640)	7,500
	Microtome, precision rotary, AO 820	4,200
Equipment leased	2 Airconditioners	700
Overhead 10% of costs, excluding equipment		5,640
		92,670

DETAILED BUDGET

(U.S. DOLLARS)
1986

Salaries : Staff (10% increase for inflation from 1985)

	Name	Title	Annual Salary	% of Time devoted to work	Salary charged to project
1.	Dr. F. Huq	Virologist	5,500	50%	-
2.	Dr. N. Shahid	Physician	4,400	50%	2,200
3.	Dr. T. Butler	Coordinator	67,000	20%	-
4.	Dr. N. Nahar	Padiatrician	4,000	10%	-
5.	Dr. M. Islam	Pathologist	33,000	10%	3,300
6.	Dr. M. Bennish	Chlamydiologist	44,000	10%	-
7.	To be named	Virology Res.Offi	3,300	100%	3,300
8.	To be named	Microb.Res.Officer	3,300	100%	3,300
9.	To be named	Technicians (3)	8,250	100%	8,250
10.	To be named	Lab Assistant (2)	5,500	100%	5,500

(Fringe benefits included in salaries)

Consultants International

	Name	Function	No. of Days	Proposed Daily rate	Total cost
1.	To be named	Ped.Pathologist	30	70	2,100
2.	To be named	Virologist	30	70	2,100

Travel International

	To Name	Number of trip	Purpose	Cost per trip	Total cost
1.	To be named	1	Consultant	2,500	2,500
2.	To be named	1	Consultant	2,500	2,500
3.	Dr. T. Butler	1	Coordinator	2,500	2,500

Materials, Services, Training

Purpose	Location	Total cost
Virology supplies	Dhaka	10,000
Microbiology supplies	Dhaka	5,000
Histology supplies	Dhaka	2,000
Radiology supplies	Dhaka	1,500

Equipment - None	-
Overhead, 10% of costs	5,605
Total:	61,655

DETAILED BUDGET
(U.S. DOLLARS)
1987

Salaries : Staff (10% increase for inflation from 1986)

	Name	Title	Annual Annual Salary	% of Time devoted to work	Salary charged to project
1.	Dr. F. Huq	Virologist	6,050	50%	-
2.	Dr. N. Shahid	Physician	4,840	50%	2,420
3.	Dr. T. Butler	Coordinator	67,000	20%	-
4.	Dr. N. Nahar	Pediatrician	4,000	10%	-
5.	Dr. M. Islam	Pathologist	36,300	10%	3,630
6.	Dr. M.Bennish	Chlamydiologist	48,400	10%	-
7.	To be named	Virol.Res.Officer	3,630	100%	3,630
8.	To be named	Microb.Res.Officer	3,630	100%	3,630
9.	To be named	Technicians(3)	9,075	100%	9,075
10.	To be named	Lab.Assistant(2)	6,050	100%	6,050
11.	To be named	Secretary	6,000	50%	3,000

(Fringe benefits - included in salary)

Consultants International - None

Travel International

Name	Number of trip	Purpose	Cost per trip	Total costs
Dr. T. Butler	1	Data analysis	3,000	3,000

Materials, Services, Training

Purpose	Location	Total costs
Virology supplies	Dhaka	3,000
Microbiology supplies	Dhaka	2,000
Histology supplies	Dhaka	1,000
Radiology supplies	Dhaka	250
Computer time	Dhaka	2,000
Publications - 2, purpose: Scientific data		800
Equipment - None		-
Overhead charge, 10% of cost		4,349
T o t a l :		47,834

SUMMARY BUDGET

(U.S. DOLLARS)

	Year 1	Year 2	Year 3	Total
Salaries:				
Existing staff	23,500	25,500	31,435	80,785
Proposed new staff	-	-	-	-
Fringe benefits	-	-	-	-
Consultants:				
Local	-	-	-	-
International	6,900	4,200	-	11,100
Travel:				
Local	-	-	-	-
International	7,500	7,500	3,000	18,000
Materials, Services, Training:				
Research expenses	18,500	18,500	8,250	45,250
Publications	-	-	800	800
Equipment:				
Leased locally	700	-	-	700
Purchased internationally	29,930	-	-	29,930
Overhead:	5,640	5,605	4,349	15,594
Total :	92,670	51,655	47,834	202,159

International Centre for Diarrheal Disease Research, Bangladesh

Informed Consent

(To be read and explained by Principal Investigator or other participating physician to parents and/or legal guardian of child to be entered into study)

Your child is ill with acute respiratory infection and will be hospitalized at ICDDR,B (or Dhaka Medical College) for treatment. Your child will be provided with the best available treatment to get full recovery from the illness. We request that you participate in a study to help us learn the causes of respiratory infection in children of Bangladesh. Your participation will be voluntary. Your child will have respiratory secretions collected by inserting a cotton swab through the nostril and by touching the back of the throat with another cotton swab. These swabs will cause minor discomfort and tickling during the collection but there is no risk of injury to your child. A sample of blood (about 1cc) will be collected by heel stick or venipuncture to allow the doctors to give better care. An x-ray (chest) will be taken. These are routine and safe procedures. In other ways, the care and medications your child will receive will be the same as for children who do not participate in the study.

Signatures will be given by the Investigator or Physician and by the parent or legal guardian. In the case that the parent or guardian is illiterate, a thumb impression of the same will be obtained.

_____
Signature of the Investigator

Date: _____

_____
Signature of the Parent / legal Guardian
(If illiterate, thumb impression)

Date: _____

আন্তর্জাতিক উদরাময় গবেষণা কেন্দ্র, বাংলাদেশ
ও
ঢাকা মেডিকেল কলেজ হাসপাতাল

অম্মতিপত্র

(প্রধান গবেষক অথবা অংশগ্রহণকারী অন্যান্য ডাক্তারেরা শিশুরোগীর মাতাপিতা অথবা অভিভাবককে এই অম্মতিপত্রটি পড়ে শুনাবেন এবং ব্যাখ্যা করে বলবেন)।

আপনার শিশু শ্বাসনালী সংক্রামক রোগে আক্রান্ত হয়েছে এবং তাকে এই উদরাময় গবেষণাকেন্দ্রে/ঢাকা মেডিকেল কলেজ হাসপাতালে ভর্তি হতে নিয়ে এসেছেন। সুচিকিৎসা দিয়ে আপনার শিশুকে সম্পূর্ন সুস্থ করে তুলতে আমরা সাহায্য করবো। আমাদের দেশের শিশুদের শ্বাসনালী সংক্রান্ত রোগের প্রধান কারনগুলো খুঁজে বের করার জন্য আমরা একটি গবেষণা কার্য্য চালাচ্ছি। আপনাকে সুইচ্ছায় আপনার শিশুকে নিয়ে আমাদের এই গবেষনায় সাহায্য করতে অনুরোধ করছি।

আপনার শিশুর নাকে ও গলার ভিতর একটি কাঠির আগায় সামান্য তুলা জড়িয়ে খুচে কিছু শ্লেষ্মা নেবো। এতে হয়ত তার সামান্য অস্বস্তি লাগতে পারে, এছাড়া কোন ক্ষতি হবে না। এর সাথে পায়ের গোড়ালী অথবা সুবিধামত শরীরের যে কোন স্থান থেকে ১ সি.সি. (1 c.c) পরিমান রক্ত নেবো পরীক্ষা করার জন্য। আপনার শিশুর বুকের এক্সরে ও নেবো। এসবগুলোই নিয়মমাফিক ও নিরাপদ মূলক পরীক্ষা। যারা এই গবেষনায় অংশ গ্রহন করছেন এবং যারা করছেন না তাদের সকলকেই একই রকম ঔষধপত্র ও একই রকম পরিচর্যা করা হবে।

ডাক্তার বা গবেষকের এবং মাতাপিতা বা অভিভাবকের সই দিতে হবে। যদি মাতাপিতা বা অভিভাবক নিরক্ষর হোন তবে টিপসই দিতে হবে।

গবেষক অথবা ডাক্তারের সই

মাতাপিতা অথবা অভিভাবকের সই

তারিখ:-

নিরক্ষর হলে টিপসই