INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH
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CENTRE
FOR HEALTH AND
POPULATION RESEARCH

PHSD
LSD
2000

MEMORANDUM

28 March 2001

To : Dr. A. K. Siddique
Public Health Sciences Division

From: Professor Mahmudur Rahman
Chairman, Ethical Review Committee (ERC)

Sub : **Approval protocol # 2000-031**

This has reference to your memo of 28th March 2001 attaching the modified version of the protocol # 2000-031 entitled "Surveillance of Dengue viral disease in Bangladesh". The protocol is hereby approved upon your addressing the issues raised by the ERC in its meeting held on 31st January 2001.

Thanking you and wishing you success in running the said study.

cc: Associate Director
Public Health Sciences Division

ICDDR,B
CENTRE
FOR HEALTH AND
POPULATION RESEARCH

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MEMORANDUM

TO: Professor Mahmudur Rahman
Chairman, ERC

FROM: Dr. A K Siddique,
Public Health Sciences Division
(P.I of Protocol # 2000-031)

DATE: 28 March, 2001

SUB: **Protocol # 2000-031**

Thank you for your memo of 7 February 2001 informing me of the decision of the ERC on the protocol # 2000-031 entitled "Surveillance of Dengue Fever in Bangladesh".

1. This is to submit that DGHS has approved implementation of the protocol in GoB Thana Health Complexes. The DGHS, however, while according permission has suggested Bajitpur instead of Iswarganj as indicated in the protocol submitted to the Committee. Copy of the letter of DGHS is attached for your kind information.

2. The consent forms have been revised to indicate possible collection of second sample as indicated on page # 12 of the protocol.

The modified copy of the protocol is attached.

I would be grateful if the protocol is approved.

Thank you.

cc: Chairman, RRC

CENTRE
FOR HEALTH AND
POPULATION RESEARCH

INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH
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MEMORANDUM

7 February 2001

To : Dr. AKM Siddique
Public Health Sciences Division

From : Professor Mahmudur Rahman
Chairman, Ethical Review Committee (ERC)

Sub : **Protocol # 2000-031**

Thank you for your protocol # 2000-031 entitled "Surveillance of Dengue viral disease in Bangladesh" which the ERC considered in its meeting held on 31st January 2001. After review and discussion in the meeting, the Committee made the following observations on the protocol:

(a) Possible collection of second sample as mentioned on page 12 should be incorporated in the consent forms.

(b) Permission of Government of Bangladesh should be obtained for conducting the study at the Thana Rural Health Centres.

You are, therefore, advised to modify the protocol incorporating the above observations and resubmit the modified proposal for consideration of the Chair.

Thank you.

cc: Associate Director
Public Health Sciences Division

ICDDRB: Centre for Health & Population Research **RRC APPLICATION FORM**

RESEARCH PROTOCOL

Protocol No.: 2000-031

FOR OFFICE USE ONLY

RRC Approval: Yes/ No Date:

ERC Approval: Yes/No Date:

AEEC Approval: Yes/No Date:

Project Title: Surveillance of Dengue viral disease in Bangladesh

Theme: *(Check all that apply)*

- ☐ Nutrition
- ☑ Emerging and Re-emerging Infectious Diseases
- ☐ Population Dynamics
- ☐ Reproductive Health
- ☐ Vaccine evaluation
- ☐ Environmental Health
- ☐ Health Services
- ☐ Child Health
- ☐ Clinical Case Management
- ☐ Social and Behavioural Sciences

Key words: dengue,

Principal Investigator: A K Siddique
Mahbubur Rahman
Abdullah H Baqui

Division: PHSD
LSD

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mahbubur@icddrb.org
abaqui@jhsph.edu

Co-Principal Investigator(s):

Co-Investigator(s):
KAHM Akram, Epidemiologist (ECPP, PHSD)
Monzoor Hussain (Director, Dhaka Shishu Hospital)
Mahamudur Rahman (Director, National Medical College Hospital)
M A Bashar, Physician (National Medical College Hospital)
S K Saha, Microbiologist (Dhaka Shishu Hospital)
M Reaz Mobarak Ali, Physician (Dhaka Shishu Hospital)

Student Investigator/Intern:

Collaborating Institute(s): GoB Health Services (3 Thana Health Complexes), Dhaka Shishu Hospital, National Medical College Hospital, Bangabandhu Sk. Mujib Medical University, AFRIMS-Bangkok, Johns Hopkins University

Population: Inclusion of special groups *(Check all that apply)*:

Gender
- ☑ Male
- ☑ Females

Age
- ☑ 0 – 5 years
- ☑ 5 – 9 years
- ☑ 10 – 19 years
- ☑ 20 +
- ☑ > 65

- ☐ Pregnant Women
- ☐ Fetuses
- ☐ Prisoners
- ☐ Destitutes
- ☐ Service providers
- ☐ Cognitively Impaired
- ☐ CSW
- ☐ Others (specify _____)
- ☐ Animal

Project / study Site *(Check all the apply)*:

- ☐ Dhaka Hospital
- ☐ Matlab Hospital
- ☐ Matlab DSS area
- ☐ Matlab non-DSS area
- ☐ Mirzapur
- ☐ Dhaka Community
- ☐ Chakaria
- ☑ Abhoynagar
- ☐ Mirsarai
- ☐ Patyia
- ☑ Other areas in Bangladesh _____
- ☐ Outside Bangladesh
  name of country: _____
- ☐ Multi centre trial
  (Name other countries involved)

Type of Study *(Check all that apply)*:

- ☐ Case Control study
- ☐ Community based trial / intervention
- ☐ Program Project (Umbrella)
- ☐ Secondary Data Analysis
- ☐ Clinical Trial (Hospital/Clinic)
- ☐ Family follow-up study
- ☐ Cross sectional survey
- ☐ Longitudinal Study (cohort or follow-up)
- ☐ Record Review
- ☐ Prophylactic trial
- ☑ Surveillance / monitoring
- ☐ Others

Targeted Population *(Check all that apply)*:

- ☐ No ethnic selection (Bangladeshi)
- ☑ Bangalee
- ☑ Tribal groups
- ☐ Expatriates
- ☐ Immigrants
- ☐ Refugee

Consent Process *(Check all that apply)*:

- ☑ Written
- ☐ Oral
- ☐ None
- ☑ Bengali language
- ☑ English language

Proposed Sample size:

Total sample size: 3456 (24 months) ☐

Sub-group 1728 from urban hospitals ☐

1728 from rural areas ☐

_____ ☐

_____ ☐

Determination of Risk: Does the Research Involve *(Check all that apply)*:

- ☐ Human exposure to radioactive agents?
- ☐ Fetal tissue or abortus?
- ☐ Investigational new device?
  (specify_____)
- ☐ Existing data available from Co-investigator
- ☐ Human exposure to infectious agents?
- ☐ Investigational new drug
- ☐ Existing data available via public archives/source
- ☐ Pathological or diagnostic clinical specimen only
- ☐ Observation of public behaviour
- ☐ New treatment regime

Yes/No

☑ ☐ Is the information recorded in such a manner that subjects can be identified from information provided directly or through identifiers linked to the subjects?

☐ ☑ Does the research deal with sensitive aspects of the subject's behaviour; sexual behaviour, alcohol use or illegal conduct such as drug use?

Could the information recorded about the individual if it became known outside of the research:

☐ ☑ a. place the subject at risk of criminal or civil liability?

☐ ☑ b. damage the subject's financial standing, reputation or employability; social rejection, lead to stigma, divorce etc.

Do you consider this research *(Check one)*:

- ☐ greater than minimal risk
- ☐ no risk
- ☐ no more than minimal risk
- ☑ only part of the diagnostic test

Minimal Risk is "a risk where the probability and magnitude of harm or discomfort anticipated in the proposed research are not greater in and of themselves than those ordinarily encountered in daily life or during the performance of routine physical, psychological examinations or tests. For example, the risk of drawing a small amount of blood from a healthy individual for research purposes is no greater than the risk of doing so as a part of routine physical examination".

Yes/No

☐ ☐ Is the proposal funded?

If yes, sponsor Name: USAID/W

Yes/No

☐ ☐ Is the proposal being submitted for funding ?

If yes, name of funding agency: (1) _____

(2) _____

Do any of the participating investigators and/or their immediate families have an equity relationship (e.g. stockholder) with the sponsor of the project or manufacturer and/or owner of the test product or device to be studied or serve as a consultant to any of the above?

IF YES, submit a written statement of disclosure to the Director.

Dates of Proposed Period of Support	**Cost Required for the Budget Period ($)**
(Day, Month, Year - DD/MM/YY)	*a. Ist Year* / *2nd Year* / *3rd Year* / *Other years*
Beginning date 01-02-2001	210,329 / 206,960 / 207,067 /
End date 3 years from start date	*b. Direct Cost :* 624,356 *Total Cost :* 774,444

Approval of the Project by the Division Director of the Applicant

The above-mentioned project has been discussed and reviewed at the Division level as well by the external reviewers. The protocol has been revised according to the reviewer's comments and is approved.

Lars Åke Persson
Name of the Division Director

Signature

05/10/00
Date of Approval

Certification by the Principal Investigator

I certify that the statements herein are true, complete and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application.

Signature of PI

Date: 25/10/00

Name of Contact Person (if applicable)

A K Siddique ECDD PMSD

Attachment 1

Date:

(FACE SHEET) **ETHICAL REVIEW COMMITTEE, ICDDR,B.**

Principal Investigator: AK SIDDIQUE

Application No. 2000-031

Title of Study: SURVEILLANCE OF DENGUE VIRAL DISEASE IN BANGLADESH.

Trainee Investigator (if any): _____

Supporting Agency (if Non-ICDDR,B) USAID/W

Project Status: _____

[✓] New Study

[ ] Continuation with change

[ ] No change (do not fill out rest of the 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) Minor or persons under guardianship — (Yes) No

2. Does the Study Involve:
   - (a) Physical risk to the subjects — Yes (No)
   - (b) Social risk — 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 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 the study — (Yes) No
   - (b) Procedures to be followed including alternatives used — (Yes) No
   - (c) Physical risk — N/A Yes No
   - (d) Sensitive questions — N/A 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 — N/A Yes No

5. Will Signed Consent Form be Required:
   - (a) From subjects — (Yes) No
   - (b) From parents or guardian (if subjects are minor) — (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 with 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. Example 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 Committee 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

Table of Contents

☐ Check here if appendix is included

ABSTRACT SUMMARY:

Globally 100 million cases of dengue fever (DF) occur annually, and over 250,000 cases of dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS) with 25,000 deaths are officially reported. Over two billion people are at risk of dengue infection around the world. DHF is one of the leading causes of morbidity and mortality predominantly in children.

The magnitude of dengue infection in Bangladesh is largely unknown. Documented evidence, however, revealed the existence of dengue viruses in Dhaka City in 1963 and in 1983. It is assumed that a significant proportion of undiagnosed febrile illness among the population of Bangladesh is associated with dengue viral infection and multiple dengue virus serotypes are co-circulating in the country particularly, in densely populated cities. Detection of dengue virus serotypes in Chittagong City in 1997 and in Dhaka City in 2000 contributes to this notion.

The present epidemiological status of dengue fever in Bangladesh demands careful monitoring. It is highly likely that DHF epidemics will continue to increase in frequency and magnitude in Bangladesh following the recent outbreak in which more than 4,000 dengue cases were hospitalized. The impact of a life-threatening epidemic disease in the community could be devastating. Introduction of epidemic of DHF/DSS would be a great threat particularly, to children of Bangladesh.

The aims of the proposed surveillance are to set up clinical and serological surveillance to

a) study epidemiology (e.g., prevalence, age distribution, clinical presentation and seasonality) of DF and DHF;
b) establish laboratory capabilities to identify dengue viral infection including primary and secondary infection and determination of serotypes involved in primary and secondary infection;
c) improve awareness, referral and treatment of severe dengue disease;
d) disseminate relevant information to professionals and to policy decision-makers.

The clinical surveillance will be conducted for a period of 24 months. Dhaka Shishu hospital and Dhaka National Medical College Hospital will be included for the study. Eight hundred and sixty four patients over 6 months of age, seeking treatment for febrile illness in each of these hospitals, will be enrolled for clinical and serological surveillance during this period. Relevant epidemiological and clinical data will be collected from patients with history of fever for 2-7 days, using standardized forms. Enrolled patients will be clinically investigated.

Laboratory investigations as indicated by clinical observations will be conducted. Blood samples (5 ml) will be collected for required laboratory tests and for dengue serological tests. Primary and secondary dengue viral infection will be detected using serological tests. Surveillance will also identify DF and DHF-DSS patients and circulating dengue virus serotypes and type-specific sequence of primary and secondary dengue infection using established clinical case definition and laboratory methods such as reverse transcriptase-polmerase chain reaction (RT-PCR) and neutralization tests.

Serological surveillance also will be conducted two times a year in four sentinel rural Thanas in Chattak, Rangamati, Iswarganj and Abhaynagar. The objective of rural surveillance is to identify circulating dengue serotypes and rate of dengue prevalence in rural areas. The surveillance period will include dry and cool months when dengue transmission is expected to be minimum and wet months when increased dengue

transmission is expected. The surveillance will be conducted for fifteen consecutive working days in each visit in each site. A total of 1,920 patients over 6 months of age seeking treatment for febrile illness will be serologically investigated. Blood (5 ml) will be collected. Serum will be tested by immunochromatic test for rapid identification of dengue infection. Suspected DHF patients will be hospitalized and appropriate treatment will be ensured. Further tests (RT-PCR) will be conducted at ICDDR, B Dhaka laboratory.

Structured questionnaire will be used for collecting data including demographic information, history of onset of illness, and clinical findings. Laboratory results and serological findings of each patient will be recorded. A designated ID number will identify each study subject. Clinical criteria established by WHO will be used for defining DF, DHF, DSS, and gradation of disease severity.

Data will be analysed to estimate:

Prevalence rate of dengue infection;
Rate of primary and secondary dengue infection;
Distribution virus sero-specific primary and secondary infection
Clinical and epidemiological factors associated with dengue positive cases such as age, gender, time of the year and severity of the disease (DF, DHF and DSS).

1. The study will be conducted among population over 6 months of age since Dengue infection occurs in all age including children. It may be mentioned that severe form of Dengue disease such as Dengue hemorrhagic fever predominantly occurs in children.

2. The subjects of the study will have no potential risk such as physical, psychological, social, legal or other as methods of the study involve routine procedures that are required for the treatment of the study patients.

3. Routine safety procedure will be followed during blood drawing and there is usually no potential risk involved. Safety measure is always effective.

4. Data generated in the study shall be kept anonymous and confidential. The raw data shall be kept under lock and key and will be available to the investigators or used under strict supervision. All computer disks or files containing data will also be maintained in a sucure place.
5. A signed consent will be obtained from the interviewee or from their accompanying relative in case of children before filling in the questionnaire and anonymity will be maintained.

6. The interview will involve the study subject or their accompanying relative in case of children in the 2 City hospitals and in the 4 Thana health complexes. This will take approximately 20 minutes.

7. The study subjects will be benefited by correct diagnosis and proper management of their illness concerned. The findings of the project will facilitate preparedness, better management and control of dengue infections by the medical professionals of the country.

8. The study will look into the medical records of hospitals providing major intervention for illness, especially information will be collected from febrile cases.

PROJECT SUMMARY: Describe in concise terms, the hypothesis, objectives, and the relevant background of the project. Describe concisely the experimental design and research methods for achieving the objectives. This description will serve as a succinct and precise and accurate description of the proposed research is required. This summary must be understandable and interpretable when removed from the main application. **(TYPE TEXT WITHIN THE SPACE PROVIDED).**

Principal Investigator: A K Siddique, Mahbubur Rahman and Abdullah H Baqui

Project Name: Surveillance of Dengue viral disease in Bangladesh

Total Budget: US$ 624,356 Beginning Date: 01.2.2001 Ending Date: 31.01.2004

Globally 100 million cases of dengue fever (DF) occur annually, and over 250,000 cases of dengue haemorrhagic fever (DHF/DSS) with 25,000 deaths are officially reported. Over two billion people are at risk of dengue infection around the world. DHF is one of the leading causes of morbidity and mortality predominantly in children, particularly, in Southeast Asian countries including Myanmar and India. Four distinct viruses that are antigenically closely related and can be identified as a serotype cause dengue infection. Infection with one dengue serotype confers only transient immunity to infection with heterologous serotypes. Moreover, infection with one serotype constitutes a risk for developing DHF/DSS during subsequent infection with other serotypes. Infants who acquire dengue antibody passively from their dengue-immune mothers are at higher risk of developing DHF-DSS during dengue infection. DHF is strongly associated with secondary dengue infection. Universally, the illness produced by dengue fever is frequently diagnosed as pharyngitis, influenza or upper respiratory tract infection and as pyrexia of unknown origin (PUO).

The magnitude of dengue infection in Bangladesh is largely unknown. Documented evidence, however, revealed the existence of dengue viruses in Dhaka City in 1963, 1983 and in 2000 when a large number of dengue cases were recognised. Many patients in the country seeking treatment for febrile illness that are clinically diagnosed as fever, viral fever or PUO is likely to be due to dengue infection. It is highly likely that multiple dengue viruses are co-circulating in Bangladesh, particularly, in densely populated cities. Detection of dengue virus serotypes in Chittagong City in 1997 and in Dhaka city in 2000 contributes to this notion. Furthermore, increased air travel contributes to the risk of introduction of Southeast Asian dengue virus genotype DEN-2, which is known for initiating DHF epidemics. We assume that a significant proportion of undiagnosed febrile illness among the population of Bangladesh is associated with dengue viral infection and multiple dengue virus serotypes are co-circulating in the country. It is highly likely that DHF epidemics will continue to increase in frequency and magnitude in Bangladesh following the recent outbreak in which nearly 4,000 dengue cases were hospitalised. The impact of a life-threatening epidemic disease in the community could be devastating. Introduction of epidemic of DHF/DSS would be a great threat particularly, to children of Bangladesh. This is an epidemiological status that demands assessment and monitoring.

The aims of the proposed surveillance are to set up clinical and serological surveillance to (a) study epidemiology (e.g., prevalence, age distribution, clinical presentation and seasonality) of DF and DHF (b) establish laboratory capabilities to identify dengue viral infection including primary and secondary infection and determination of serotypes involved in primary and secondary infection; (c) improve awareness, referral and treatment of severe dengue disease; and (d) to disseminate relevant information to professionals and to policy decision-makers.

The clinical surveillance will be conducted for a period of 24 months. Dhaka Shishu hospital and Dhaka National Medical College Hospital will be included for the study. Eight hundred and sixty four patients over 6 months of age, seeking treatment for febrile illness in each of these hospitals, will be enrolled for clinical and serological surveillance. Relevant epidemiological and clinical data will be collected from patients with history of fever for 2-7 days, using standardised forms. Enrolled patients will be clinically investigated. Laboratory investigations as indicated by clinical observations will be conducted. Blood samples (5 ml) will be collected for required laboratory tests and for dengue serological tests. Primary and secondary dengue viral infection will be detected using clinical criteria and rapid serological tests. Surveillance will also identify DF and DHF-DSS patients and circulating dengue virus serotypes and type-specific sequence of primary and secondary dengue infection using established clinical case definition and laboratory methods such as reverse transcriptase-polmerase chain reaction (RT-PCR) and neutralisation tests. Laboratory methods will be periodically assessed at the reference laboratory to ensure quality.

Serological surveillance also will be conducted at the four sentinel rural Thanas in Chattak, Rangamati, Iswarganj and Abhaynagar. The objective of rural surveillance is to identify circulating dengue serotypes in rural areas. The surveillance will be conducted for fifteen consecutive working days, two times a year in each site. A total of 1,920 patients over 6 months of age seeking treatment for febrile illness will be enrolled in 24 months. Blood (5 ml) will be collected. Serum will be tested by immunochromatic test for rapid identification of dengue infection. Suspected DHF

patients will be hospitalised and appropriate treatment will be ensured. Further tests (RT-PCR and ELISA) will be conducted at ICDDR, B Dhaka laboratory.

Dissemination of the findings of the project will facilitate better preparedness and better management of patients. Technical assistance by the project will contribute to building capacity of the national institutions and also contribute to develop policies by the decision-makers. Mechanism of co-ordination with the government of Bangladesh (GoB) will be developed. The epidemiological findings of the study will also contribute to the global effort to understand the mechanism of life-threatening epidemics of DHF.

KEY PERSONNEL (List names of all investigators including PI and their respective specialties)

Name	Professional Discipline/ Specialty	Role in the Project
1. A K Siddique	Epidemiologist (Head, ECPP, PHSD)	Principal Investigator
2. Mahbubur Rahman	Microbiologist, (ARI Lab, LSD)	Principal Investigator
3. Abdullah H Baqui	Epidemiologist (Johns Hopkins University, MD, USA)	Principal Investigator
4. KAHM Akram,	Epidemiologist (ECPP, PHSD)	Co-Investigator
5. Monzoor Hussain	(Director, Dhaka Shishu Hospital)	Co-Investigator
6. Mahamudur Rahman	(Director, National Medical College Hospital)	Co-Investigator
7. M A Bashar	Physician (National Medical College Hospital)	Co-Investigator
8. S K Saha	Microbiologist (Dhaka Shishu Hospital)	Co-Investigator
9. M Reaz Mobarak Ali	Physician (Dhaka Shishu Hospital)	Co-Investigator

DESCRIPTION OF THE RESEARCH PROJECT

Hypothesis to be tested:

Concisely list in order, in the space provided, the hypothesis to be tested and the Specific Aims of the proposed study. Provide the scientific basis of the hypothesis, critically examining the observations leading to the formulation of the hypothesis.

1. A significant proportion of undiagnosed febrile illness among the population particularly, in children, of Bangladesh is associated with dengue viral infection.
2. Multiple dengue virus serotypes are co-circulating in Bangladesh largely producing unrecognised Dengue haemorrhagic diseases

Specific Aims:

Describe the specific aims of the proposed study. State the specific parameters, biological functions/ rates/ processes that will be assessed by specific methods (TYPE WITHIN LIMITS).

1. To set up clinical and serological surveillance in selected urban and rural health facilities to study the epidemiology (e.g., prevalence, age distribution, clinical presentation and seasonality) of Dengue Fever (DF) and Dengue Haemorrhagic (DHF-DSS).
2. To establish laboratory capabilities to recognise dengue viral infection and to determine the virus serotypes involved in primary and secondary infection using established diagnostic methods;
3. To improve awareness, referral and treatment of severe dengue disease;
4. To disseminate relevant information to professionals and policy decision-makers.

Background of the Project including Preliminary Observations

Describe the relevant background of the proposed study. Discuss the previous related works on the subject by citing specific references. Describe logically how the present hypothesis is supported by the relevant background observations including any preliminary results that may be available. Critically analyze available knowledge in the field of the proposed study and discuss the questions and gaps in the knowledge that need to be fulfilled to achieve the proposed goals. Provide scientific validity of the hypothesis on the basis of background information. If there is no sufficient information on the subject, indicate the need to develop new knowledge. Also include the **significance and rationale** of the proposed work by specifically discussing how these accomplishments will bring benefit to human health in relation to biomedical, social, and environmental perspectives. (DO NOT EXCEED 5 PAGES, USE CONTINUATION SHEETS).

Dengue which is one of the most important arbovirus disease in man, has re-emerged as a major cause of morbidity and mortality in the tropics and sub-tropics (WHO 1993) inhabited by more than two thirds of people living on earth. Over 2.5 million cases of DHF (Dengue Haemorrhagic Fever) and 40,000 deaths were officially reported between 1956 and 1990 in south-eastern Asia including the Indian sub-continent, which is one of the vulnerable areas for dengue haemorrhagic fever epidemics (Halstead 1992). Globally, an estimated 100 million cases of dengue fever (DF) occur annually and over 250,000 cases of DHF and DSS (Dengue Shock Syndrome) are officially notified (Monath 1994). At present, specific treatment against the disease is neither available, nor are there effective vaccines. Untreated case fatality rates of severe disease could be as high as 30-40% (WHO 1997).

Infection with a dengue viruses may be clinically inapparent or may produce nonspecific febrile illness, classical dengue fever (DF) or dengue haemorrhagic fever (DHF). Classical dengue fever is characterised by fever, malaise, headache, arthralgia, myalgia and rash. In the early febrile phase, DHF is indistinguishable from DF. The major clinical manifestations that characterise a typical case of DHF are high fever, haemorrhagic phenomena, thrombocytopenia and circulatory failure (due to plasma leakage) that may lead to life threatening dengue shock syndrome (Halstead 1969, 1980; Kuberski 1977; Nimmannity 1969; WHO 1997).

The South East Asian region known as the "home" of dengue viruses (Rudnick 1978) had only sporadic outbreaks of dengue before World War II. In the two decades, during and after the war, epidemic dengue spread to Islands in the sea of Japan (Sabin 1952), Pacific Islands, Vietnam, Malaysia, Singapore, Indonesia, Southern American countries (PAHO. 1979; Gubler 1987; Figueriredo 1990; Pinheiro 1989; Irving 1992).

Classical dengue fever (DF) affects all age groups. During an outbreak of DF in Maharashtra, India, in 1988, 70% of the population affected were adults (Mehendale 1991). The most dramatic change in dengue transmission in recent decades has been the emergence of epidemics of Dengue Haemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS), which is the most severe form of the disease. DHF is now recognised as one of the leading causes of childhood illness (WHO 1993). Dengue Haemorrhagic Fever (DHF) was first reported in the Philippines, in 1954 (Quintos 1954). Two years later an outbreak resulted in over 2500 hospitalisation of cases with nearly 10% case-fatality (Hammon 1960; Bhamarapravati 1962). Between 1958 and 1963, nearly all (90%) of the 10,367 cases and 649 deaths reported in Bangkok were children below 14 years of age (Halstead 1970). Deaths due to dengue occur more frequently in females than males (Halstead 1970). In 1981, for the first time in the Americas, an epidemic of DHF was reported in Cuba in which more than 24,000 patients with severe haemorrhagic symptoms and nearly 10,000 cases of DHF/ dengue shock syndrome was reported (Kerschner 1986; Kouri 1989; Lewis 1993). A total of 116,000 patients were hospitalised over a period of three months during the epidemic (Guzman 1984).

Dengue virus, for the first time in the Indian-subcontinent, was isolated in Calcutta in 1945 (Sabin 1952). However, the first DHF epidemic in India was reported in Calcutta in 1963 (Sarkar 1964; Ramakishnan 1964). In 1988, a large epidemic of DHF/DSS occurred in Delhi city in which nearly 30% of the population of the city were affected (Acharya 1988). In one of the children's hospital the case-fatality of DHF/DSS was over 33 % (Kabra 1992). The largest DHF epidemic in India, however, was reported in 1996 (Mudur 1996). Recurrent epidemics of dengue have ocurred in Myanmar since 1963 (Ming 1974; Thaung 1975). During the epidemic in 1970, over 1600 patients with haemorrhagic fever were hospitalised in Rangoon (Ming 1974). Between 1956 and 1990 nearly 60,000 cases of dengue haemorrhagic fever (DHF) have been reported in Myanmar (Halstead 1992).

The central facts of ecology of dengue fever are the four distinct viruses (DEN 1-4) which are antigenically closely related and responsible for producing the disease. They are transmitted only by certain species of day-biting *Aedes* mosquitoes, particularly, *Aedes aegypti* and that human beings and in some regions monkeys, constitute the cycles of infection by which the virus is perpetuated (Rudnick 1965). The virus survives in nature by two mechanisms; by

transmission between infected vertebrate and mosquitoes and by vertical transmission in the mosquito. *Aedes aegypti* predominantly breeds indoors, in clean stored water, in ceramic jars or metal drums, water-holding planters and outdoors in natural or artificial containers which trap rain water such as rubber tires, tin cans, plastic cups, bamboo internodes, coconut shells, etc (Gould 1968). *Aedes aegypti* mosquito can acquire the infection from febrile patients. Once infective a mosquito can serve as a vector for the rest of its life (approx. 2-3 weeks). A high degree of anthropophilia, multiple and interrupted blood feeding habits (Macdonald 1956), and the urban location of breeding makes A. *aegypti* a highly efficient vector.

Aedes aegypti has a short (50-100 yards) flight range (Reiter 1995). They seldom disperse more than a few hundred yards from their place of birth (Sheppard 1969). The geographical dispersion of dengue viruses is largely by the movement of viremic human beings. The "Jet age" air travel facilitates the quick geographic dispersion of dengue. An infected index case introduces viruses into a household infested with vector mosquitoes resulting in secondary cases (Gubler 1976). Crowded urban areas therefore, provide ideal opportunities for dengue transmission.

Studies in India and Thailand show that temperature fluctuation between the normal summer and winter is correlated with dengue transmission (Carey 1966; Halstead 1969; Burke 1980). Dengue endemic areas with mean temperature of 36^0C during the rainy season have four times higher risk of dengue transmission compared to areas with mean rainy season temperature of 17^0C (Koopman 1991). High temperature increases vector efficiency by reducing the period of viral replication in mosquitoes (Watts 1987). The El Nino Southern Oscillation (ESNO), which is a global scale pattern of climate variation, recurring over a 2-7 year cycle, greatly influences the temperature and rainfall. ESNO seems to influence temperature and rainfall sensitive vector-borne disease (WHO 1990;Nicholls 1993; Bouma 1996). A positive correlation between Southern Oscillation Index (ISO) and dengue fever epidemics has been shown (Hales 1996). The Inter-governmental Panel on Climate Change (IPCC) warned that if global warming is accompanied by increased precipitation, there could be an increase in the incidence of dengue in many countries (IPCC 1990).

For decades, attempts have been made to control *Aedes aegypti* population using the ultra-low volume (ULV) aerosol spray of technical grade insecticides to kill adult mosquitoes (Gratz 1991). However, studies in Thailand, Jamaica and Venezuela have shown that mosquito population reappears to pre-treatment levels within a short period (Pant 1971;Chadee 1985; Hudson 1986; Focks 1986;Newton 1992). Resistance to insecticides by *Aedes aegypti* seems to limit success in controlling its population.

Dengue viron is a single-strand genome. Each dengue virus can be identified as a serotype by type specific determinants. Unlike most of the infectious diseases, prior infection with one dengue serotype (DEN-1, DEN-2, DEN-3, and DEN-4) confers only transient immunity to infection with heterologous serotypes (Sabin 1952). However, it does not give rise to antibodies broadly cross reactive with virons of other serotypes. Therefore, individuals infected with one serotype are susceptible to infection with the three other serotypes. Dengue appears to have evolved as parasite to mononuclear phagocytes (Dasancyavaja 1961: Nisalak 1970: Sumarmo 1983; Yoksan1984). Peripheral-blood leucocytes are the target cells for the dengue viruses and their permissiveness to infection correlates with presence of humoral antibodies (Marchette 1975,1976:Halstead 1976). In culture of human monocytes dengue virus replicates in higher titres in presence of antibody at sub-neutralising level than in culture without antibody (Halstead 1973,1977).

Prior dengue infection constitutes a risk of developing dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS) during subsequent infection (Hammon 1963;Halstead 1986). This unique phenomenon in human disease has been attributed to a complex immunopathological mechanism produced by antibody dependent enhancement of infection (ADE). Pre-existing cross-reactive, non-neutralising antibodies (IgG isotype) of a heterologous dengue serotype form immune complexes with virons of the current (secondary) infection and bind with FCγ receptors of monocytic-macrophages enhancing viral replication in these cells (Halstead 1986). Infected mononuclear phagocytes are activated that in turn triggers activation of complement system with release of thromboplastin and macrophage-generated vascular permeability factors which possibly contributes to pathology of haemorrhage and shock (Russsell 1969;Bokishch 1973;Nishoka 1975; Phanichyakaran1972, 1977). There is increased T-cell activation and introduction of cross-reactive CD4 and CD8 cytotoxic T-cells that recognise dengue viral antigens on infected monocytes. Though the process is the key to clearance of infected cells (Kurane 1992) and recovery of the host from infection, however, this may also have pathological consequences due to release of cytokines with vasoactive or procoagulant properties, and release of interferon γ which upregulates expression of FC receptors contributing to enhancement of viral replication (Monath 1994) The larger the number of cells infected with dengue viruses, the more activation products are generated and released (Halstead 1980).

Infants who acquire passive dengue immunity from their dengue-immune mothers seem to be at higher risk of developing DHF/DSS during an initial dengue infection. Such immunological groups were identified in Thailand, Myanmar, Indonesia, Cuba and Puerto Rico (Halstead 1968; Sumarmo 1983; Guzman 1984). The incidences of DHF

in infants are most frequent between the age of 6 and 8 months (Halstead 1969). This may represent the time required for catabolism of protective antibody acquired at birth. DHF in children beyond infancy is strongly associated with secondary dengue infection. Studies in Thailand showed that over 90% of DHF/DSS patients were one year old or older children and nearly all (99%) had a previous dengue infection (Halstead 1969; Sangkawibha 1984).

The orders of primary and secondary infections affected by particular DEN virus seem to determine the degree of severity of the disease (Sangkawibha 1984). The high case fatality in Athens was attributed to the sequences of dengue infections by DEN-1 in 1927 followed by serotype DEN-2 in 1928 (Papaevangelou 1964; Halsted 1965). In Tahiti, infection of DEN-3 was followed by DEN-2 during DHF epidemics in 1971 (Moreau 1973). Studies in Thailand showed that highest relative risk of DSS was associated with the infection sequences of DEN-1 and DEN-2 (Sangkwibha 1984; Burke 1988). Infection sequence ending with DEN-2 virus type was attributed to higher risk of DSS.

DHF epidemics have also been associated with geographic areas where more than one DEN virus co-circulates (Gubler 1994). Molecular studies indicated that DEN-1 isolated in America was introduced from Africa in 1977 (PAHO 1979). Of the two different genotypes of DEN-2 that are currently circulating in the American region, the one that was first isolated in Trinidad in 1953 (Anderson 1956) is genetically related to DEN-2 virus from India and Pacific (Lewis 1993;Rico-Hesse 1990; Trent 1983,1990). The genotype, possibly, was introduced to the Americas from the Indian sub-continent. The new genotype of DEN-2 that was responsible for the first epidemic of DHF in Cuba and later in Jamaica (Gubler 1987), is genetically related to DEN-2 viruses isolated in Thailand, and Vietnam (Lewis 1993; Rico-Hesse 1990). Cubans working in Vietnam may have brought the genotype home.

Dengue viruses, which had been associated with non-fatal epidemic disease for more than 100 years have, in recent decades, transformed into causing fatal disease in the form of Dengue haemorrhagic fever (DHF) in many countries. Not very clearly understood is the difference between dengue viruses, which causes DHF/DSS in the presence of pre-existing antibody and those, which do not (Monath 1994). Multiple dengue virus serotypes existed in Americas for many years before the first DHF epidemic started in Cuba in 1981 (WHO 1993). Despite the existence of all four dengue viruses, epidemics of DHF in India were rare prior to 1988. In recent years epidemic of DHF in India has turned into a major public health threat. Over 47,000 patients were hospitalised and at least 220 patients died during the 1996 DHF epidemic in Delhi, India. The reason for the dramatic change in dengue epidemiology in India since 1988 is not clear. Whether the change was due to introduction of the Southeast Asian genotype DEN-2 as observed in Cuba in 1981 (Ricco-Hesse 1990), or due to emergence of a new biotype with increased virulence are some of the questions that remain unresolved. The impact of a life-threatening epidemic disease in an unprepared community could be devastating. Inadequate surveillance, lack of appropriate laboratory facilities, lack of trained health-care providers and absence of public health research programme is thought to have contributed to the vulnerability of the population of India to DHF epidemics (Mudur 1996).

Significance of Dengue Fever in Bangladesh

Universally, the illness produced by classical dengue fever is more frequently diagnosed as pharyngitis, influenza or upper respiratory tract infection (Halstead 1969). Studies in Thailand showed that 60% of children admitted in hospital with clinical diagnosis of pyrexia of unknown origin (PUO) that had virological and serological evidence of dengue infection (Nimmannitya 1969). This may also be applicable to Bangladesh. Nation-wide reliable data relating to febrile illness is lacking. Data from four thana (sub-districts) health facilities providing health care for nearly 1.7 million people indicated that over 15 % patients at thana health Centres per year were clinically classified as fever, viral fever or PUO (Siddique 1997:Unpublished data). A longitudinal survey in Dhaka City (pop.8 million) revealed that common cold with fever (21%) and fever (18%) were the two most frequent categories of illness (Desmet 1997).

The current prevalence of dengue viral disease or about the serotype of dengue viruses circulating in Bangladesh is largely unknown. However, during a large febrile epidemic in Dhaka in 1963 (Dhaka Fever), blood samples tested for dengue infection was positive for dengue virus type DEN-3 (Russell 1966). In 1983 a sample of blood from school children tested against dengue infection were positive for DEN-1 virus (Khan 1986). This could be true for other areas of the country: a hospital study in Chittagong City (1997) revealed the presence of DEN 2-4 virus serotypes (Yunus 1997). In recent years the population density of *A. aegypti* in Dhaka city has increased significantly (Dept. of Entomology GoB: Personal communication). Therefore, introduction of dengue viruses, having epidemic potential, in these settings would greatly increase the likelihood of DHF epidemics. This assumption was proven true in June 2000 when an outbreak of DHF occurred for the first time in Bangladesh when nearly 4,000 suspected dengue cases were hospitalised. In one hospital in Dhaka City, 69 of the 80 suspected dengue fever patients had serologically dengue infection (as determined by serum anti-dengue IgM and IgG capture ELISA). The disease patterns indicated 66.7% were DF, 32% DHF and 1.45% cases were DSS. Secondary infection (IgM/IgG ratio < 1.8) was detected in 78% of

cases. Dengue virus serotype 3 (DEN-3) was detected in 5 of the 20 patients by RT-PCR, 4 of them had DHF.

A consistent observation in most of the DHF epidemic affected areas is the presence of multiple circulating DEN serotypes and occurrence of smaller DHF outbreaks over a period of time followed by severe epidemics. This trend has also been observed in India. It is also likely that Bangladesh is having a similar situation. This is an epidemiological status that merits careful monitoring. It is highly likely that DHF epidemics will increase in frequency and in greater magnitude in Bangladesh in the near future. The serious form of the disease (DHF/DSS) would be a great threat particularly to children of Bangladesh. Introduction of DHF epidemics will worsen the existing high child morality rate. The proposed surveillance will provide epidemiological data, which will contribute to better preparedness and management of patients. It will also contribute to understanding of the mechanism of life-threatening epidemics of DHF/DSS that has emerged as a global health problem.

Research Design and Methods

Describe in detail the methods and procedures that will be used to accomplish the objectives and specific aims of the project. Discuss the alternative methods that are available and justify the use of the method proposed in the study. Justify the scientific validity of the methodological approach (biomedical, social, or environmental) as an investigation tool to achieve the specific aims. Discuss the limitations and difficulties of the proposed procedures and sufficiently justify the use of them. Discuss the ethical issues related to biomedical and social research for employing special procedures, such as invasive procedures in sick children, use of isotopes or any other hazardous materials, or social questionnaires relating to individual privacy. Point out safety procedures to be observed for protection of individuals during any situations or materials that may be injurious to human health. The methodology section should be sufficiently descriptive to allow the reviewers to make valid and unambiguous assessment of the project. (DO NOT EXCEED TEN PAGES, USE CONTINUATION SHEETS).

The surveillance project will have the following components: (1) Clinical surveillance for detectation of dengue fever (DF) and dengue haemorrhagic fever (DHF/DSS); (2) Serological surveillance to identify circulating dengue serotypes and to determine sero-specific sequence of primary and secondary dengue infection from specimens collected by the clinical surveillance; (3) Dissemination and policy recommendation

1. Study population, setting, and subjects

1.1. Reference Population:

Febrile patients over 6 months of age from the:

(a) catchment areas of two general Hospital of Dhaka city.
(b) rural health facilities of Chattak, Iswarganj ,Rangamati sadar and in Abhayanagar Thana.

1. 2. Population source:

Patients seeking treatment for febrile illness at (a) Dhaka Shishu Hospital, (b) Dhaka National Medical College Hospital and (c) Thana health complexes of Chattak, Rangamati and Iswarganj.

Dhaka Shishu Hospital is the largest paediatric hospital of the country. The 350 bed hospital provides outpatient and in-patient care. The inpatient facility includes different specialities e.g., nutrition rehabilitation, diarrhoea, developmental paediatrics, neonatology, surgery etc. The laboratory facilities include routine microscopy, biochemistry and selected serology. Annually, nearly 150,000 sick children attends the outpatient department The hospital caters treatment to patients from Dhaka city as well as from other parts of the country with both acute and chronic conditions. Majority of the inpatient beds is free so that the poor can access the services. About 10% of the patients seen in the OPD are admitted in different units of the hospital. The hospital is managed partly through governmental funding. It also generates some income from service fees and receives donations from individuals and organisations.

The National Medical College Hospital has 300 beds. The hospital provides services for over 500,000 city dwellers that include outpatient clinics and comprehensive in-patient care, emergency care, laboratory services, X-ray and also intensive care. Between 160,000 and 170,000 patients utilise the outpatient facilities of the hospital annually. The laboratory facilities include routine microscopy, biochemistry and selected serology.

The Thana health complex is a government run rural heath facility that offer outpatient services in-patient care as well as primary heath care for approximately 200,000 population. The Epidemic Control Preparedness Programme (ECPP) currently conducting cholera surveillance in all these sites with the exception of Rangamati. Data collected by ECPP revealed that between 10 and 15 febrile patients designated as PUO seek treatment at the out patient department in Thana health complexes on each working day. Analysis of distribution by months indicated that most of cases occurred from May through October.

1. 3. Sample size estimation:

In a hospital based survey in Chittagong, it was observed that 14% of 225 febrile patients enrolled were dengue positive (Yunus 1997). Studies in dengue prevalent areas have revealed that over half of the children with PUO had serological evidence of dengue infection (Halstead). We assume that about 10% of fever cases seeking treatment at the health facilities in Dhaka City and in other smaller urban or semi-urban areas are likely due to dengue infection.

Dengue Prevalence	Precision	Required Sample Size
.05 (5%)	± 20% (range .04 - .06)	1,238
0.1 (10%)	**± 20% (range .08 - .12)**	**864**
0.15 (15%)	± 20% (range .12 - .18)	544

To estimate a prevalence of 10% with precision of ±20%, a sample of 864 is required. We would like to have separate annual estimate for urban and rural areas. Therefore, we will need to study 864 cases per year from the two urban hospitals, and another 864 cases per year from the four rural sentinel health facilities. If four eligible subjects are enrolled on alternate days in each of the Dhaka city hospitals (240 working days/year), then 1,728 subjects will be enrolled in 24 months, which is adequate for the estimated sample size with a precision of ±20%.

The surveillance at each of the four rural sentinel sites will be conducted two times a year. During each visit the surveillance will be conducted for fifteen consecutive working days (total of 120 day/year). If eight eligible subjects are enrolled in each surveillance day from the four surveillance site, then a total of 1,920 will be enrolled in 24 months, which will meet the required sample size.

2. Surveillance methods

2.1. Clinical surveillance

Febrile patients over 6 months of age seeking care in hospitals will be studied to identify DF, DHF/DSS using clinical criteria set up WHO and established laboratory diagnostic methods.

Eligibility for enrolment:

Inclusion Criteria

- Age: Over 6 months
- Illness: History of fever for 2-7 days

Exclusion Criteria

- Refuses to be enrolled
- Refuses blood tests
- Specific identifiable focal cause of fever

Case definition:

The WHO (WHO 1986) criteria will be adopted for clinical definition of dengue fever (DF), dengue haemorrhagic fever (DHF) and Dengue Shock Syndrome (DSS)

Dengue fever (DF):

Acute febrile illness with two or more of the following manifestations:

- Headache
- retro-orbital pain
- Myalgia
- arthralgia
- rash
- haemorrhagic manifestations
- leucopenia

plus- detectable IgM or IgG as indicated by immunochromatographic rapid test

Dengue Haemorrhagic fever (DHF):

The followings must be present:

- Fever for 2-7 days (occasionally biphasic)
- Haemorrhagic tendencies, evidenced by at least one of the following:
  --petechiae, ecchymoses or purpura
  -- bleeding from the mucosa, gastrointestinal tract, injection sites or other locations and
  --a positive tourniquet test[1]

- Thrombocytopenia (100000 cells per mm^3 or less)[2]
- A rise in the haematocrit ≥ 20% above (determined by capillary method) average for age will be considered as the evidence of plasma leakage due to increased vascular permeability

Plus - detectable IgG/IgM and IgG as indicated by immunochromatographic rapid test

Dengue shock syndrome:

All of the above criteria for DHF must be present, plus evidence of circulatory failure manifested by:

- Rapid and weak pulse, and
- Narrow pulse pressure (<20 mm Hg), hypotension for age,[3]
- Cold, clammy skin and restlessness.

2.2. Case detection, collection of specimens

Dhaka city hospitals:

The first four febrile patients, at the outpatient department of each study hospital, meeting the eligibility criteria will be enrolled for surveillance on each working day. Informed consent will be obtained from patient or parents/caretakers. A study physician will examine all enrolled patients and will interview the patient or parents/caretakers to collect demographic and clinical information using a structured questionnaire. Detailed address of the subject will also be noted.

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[1] The tourniquet test will be performed by inflating a blood pressure cuff on the upper arm to a point midway between the systolic and diastolic pressures for 5 minutes. A test is considered positive when 20 or more petechiae per 2.5 cm square are observed. The test may be negative or mildly positive during the phase of profound shock. It usually becomes positive, sometimes strongly positive, if the test is conducted after recovery from shock.

[2] This number represents a direct count using a phase-contrast microscope (normal is 200,000–500,000 per mm^3). In practice, for outpatients, an approximate count from a peripheral blood smear is acceptable. In normal persons, 4-10 platelets per oil-immersion field (100X; the average of the readings from 10 oil-immersion fields is recommended) indicates an adequate platelet count. An average of ≤3 platelets per oil-immersion field is considered low (i.e. <100,000 per mm^3).

[3] Hypotension is defined to be a systolic pressure <80 mm Hg for those less than 5 years of age, or <90 mm Hg for those greater than or equal to 5 years of age. Note that narrow pulse pressure is observed early in the course of shock, whereas hypotension is observed later, or in patients who experience severe bleeding.

The clinical examination will involve clinical history and a thorough physical examination. Laboratory investigations as indicated by clinical observations will be conducted. Blood samples (5ml) will be collected for required laboratory tests and for dengue serological tests. The study physician will review the clinical manifestations and results of laboratory tests. Patients with clinical manifestation suggestive of DHF and with detectable anti-dengue antibodies (IgG/IgM) will be hospitalised as indicated. The study physician will carefully monitor the patient for signs of impending circulatory shock and ensure appropriate treatment.

Surveillance at sentinel sites:

The purpose of rural sentinel surveillance is to identify circulating dengue serotypes in rural areas. The surveillance will be conducted two times a year in four sentinel sites. The surveillance period will include the cool and dry month when dengue transmission is expected to be minimum and, wet months when increased dengue transmission is expected. During each visit, eligible patients seeking treatment at the outpatient department of the selected Thana health complex for acute febrile illness will be enrolled for serological surveillance for fifteen consecutive working days. A visiting project physician after obtaining informed consent from patient or parents/caretaker will examine all enrolled patients and interview the patient or parents/caretakers to collect demographic and clinical information using a structured questionnaire. The clinical examination will involve clinical history and a thorough physical examination. Blood (5 ml) will be collected by venipuncture with syringe and needle. Serum samples will be tested immediately by immunochromatic test for rapid identification of dengue infection. The rest of the serum will be stored and transported, using cold chain, to ICDDR, B where it will be preserved at -72° C until use. Suspected DHF patients will be hospitalised as indicated and monitored by the study physician and appropriate treatment will be ensured.

3. Case management

The cases will be managed according to World Health Organization (WHO) recommended standard guidelines.

4. Laboratory methods for detection of dengue infection

Serum samples will be tested by rapid immunochromatographic PanBio (PanBio, Brisbane, Australia) dengue test immediately after collection. A second test will be repeated after 5 days for those patients who will be admitted in the ward and had negative result in the first test. Sera collected within 5 days of fever will be tested by reverse transcriptase-polymerase chain reaction (RT-PCR) for detection of dengue viruses and serotyping. Samples positive by RT-PCR will be cultured for isolation of dengue viruses in C6/36 cell lines.

A large proportion of dengue viral infection may remain clinically inapparent or may produce minor illness, which is self-limiting. However, secondary dengue infection can result in more serious conditions such as dengue haemorrhagic fever and dengue shock syndrome. The most challenging problem of management of patients with DHF/DSS, which is often associated with high case fatality, is the rapid laboratory supported diagnosis. Laboratory diagnosis of dengue infection can be achieved either by detection of the virus by culture or by detection of anti-dengue antibodies. Laboratory procedures for detection dengue infection and justification for selection of methods for our surveillance are mentioned in detail in the laboratory component of the protocol **(annex-2)**.

4.1. Immunochromatographic Test.

Rapid Identification of dengue infected patients

Dengue rapid test (PanBio, Brisbane, Australia) will be used to detect dengue virus-specific IgM and IgG antibodies in serum samples from suspected patients as suggested by the company. The rapid test demonstrated 100% sensitivity and specificity in the diagnosis of dengue virus infections (Vaughn 1998; Palmer 1999). A drop of serum will be added to the test pad. The serum migrates along the nitrocellulose membrane where IgG and IgM antibodies are captured by anti-human IgG and IgM striped onto the membrane. At the same time gold-labelled anti-dengue monoclonal antibody (mAb), striped onto a separate pad, will be rehydrated by the adding 2 drops of buffer. After the serum reached the marked limit line (<2 min.), the card will be closed. The rehydrated gold-labelled anti dengue mAb forms complex with dengue antigens stabilised in a pad at the top of the nitrocellulose membrane and allows binding of the gold-complexed antigen to the bound dengue-specific IgM or IgG. After 5 min. the assay results will be visible through the window on

the front pad of the card. Captured gold-labelled antigen-antibody complexes will appear as maroon lines. The results will be interpreted (according to manufacturer's direction) as:

Primary dengue infection: visible IgM and control line but not IgG line as the cut-off has been set to differentiate primary and secondary infection

Secondary dengue infection: visible IgG, IgM and control lines. Some cases of secondary infection do not show reaction to IgM line. Secondary dengue infection should be suspected when IgG and control lines are visible.

4. 2. Detection of serum antidengue IgM and IgG antibodies by ELISA

Serum samples from clinical surveillance collected between 2 and 7 days after onset of fever and tested for antidengue IgM and IgG antibodies by commercial capture ELISA (Innis 1989) by PanBio. A second serum sample will be collected after 14 days of fever for rising antibody titre whenever available. ELISA will be performed on every fourth serum sample.

4. 3. Reverse transcriptase-polymerease chain- reaction (RT-PCR) for detection and typing of dengue viruses from clinical samples and differentiation from other Arboviruses infection

Clinical samples. Serum sample will be used for extracting RNA for RT-PCR for amplification of dengue virus RNA.

RNA extraction from serum samples. Viral RNA will be extracted from serum samples by the method described by Lanciotti et al. (Lanciotti 1992). Briefly, 20 µl of serum sample will be vortexed for 5 sec with equal volume of 8 M guanidine isothiocyanate lysing buffer containing 50 mM sodium citrate, 10 mM 2-mercaptoethanol, 1% Sarkosyl and 1 µg of yeast tRNA/ml. The solution will be sequentially mixed with 1/10 volume of 2 M sodium acetate (pH 4), an equal volume of water-equilibrated phenol and a 2/10 volume of chloroform. The mixture was centrifuged at 16,000 x g for 15 min and aqueous phase will be removed and combined with equal volume of isopropanol to precipitate the RNA. After centrifugation, the resulting RNA pellet will be washed with 75% ethanol and dissolved in diethyl polycarbonate-treated water.

Selection and synthesis of primers. Dengue virus consensus primers that are able to detect all 4 serotypes published by Lanciotti et al. (Lanciottio 1997) will be used in the study to detect dengue virus and type them. Primers will be purchased from commercial source.

RT-PCR for amplification of dengue virus RNA. Target viral mRNA, extracted from plasma sample, will be converted in to cDNA by reverse transcriptase and dengue virus downstream consensus primer (D2), homologous to the genomic RNA of the 4 serotypes by standard protocol (Lanciotti 1992). Subsequent *Taq* polymerise amplification will be performed on the resulting cDNA with upstream dengue virus consensus primers (D1) by mixing all the appropriate components of RT-PCR in 100-µl reaction volume in a tube and incubating the reaction tube as described by Lanciotti (Lanciotti 1992). A nested PCR will be performed in another tube using D1 and 4 (T1, T2, T3 and T4) dengue virus type-specific primers described by Lanciotti (Lanciotti 1992). Bands of 482-bp, 119-bp, 290-bp and 392-bp, visible by agarose gel electrophoresis, will indicate dengue virus type 1, 2, 3 and 4 infection.

Screening of RT-PCR products. The RT-PCR products will be screened by 2.2% agarose gel electrophoresis followed by ethidium bromide staining.

The tests will be carried out at the ICDDR, B laboratory.

4. 4. Neutralisation test

The test will be conducted in the collaborating reference laboratory (Department of virology, Armed Forces Research Institute of Medical Sciences [AFRIM], Bangkok, Thailand. The objective of the test is to determine serospecific sequence of primary and secondary dengue infection.

C. Genotyping of Dengue Virus:

RT-PCR product will be sequenced for determining genotyping of circulating viruses during the study period.

Quality Assurance of laboratory Methods:

Laboratory methods will be assessed at regular intervals. All positive samples and 10% of the negative samples will be sent to Armed Forces Research Institute of Medical Sciences (AFRIMS) the reference for quality assurance.

Case Assignment:

Subjects with clinical and serological (as indicated by rapid immunochromatographic test) evidence of dengue virus infection will be assigned a final diagnosis DF or DHF or DHF/DSS on the basis of a review of clinical and laboratory data (including RT-PCR and Neutralisation test results) by two study physicians.

Facilities Available

Describe the availability of physical facilities at the place where the study will be carried out. For clinical and laboratory-based studies, indicate the provision of hospital and other types of patient's care facilities and adequate laboratory support. Point out the laboratory facilities and major equipments that will be required for the study. For field studies, describe the field area including its size, population, and means of communications. (TYPE WITHIN THE PROVIDED SPACE).

The International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) has facilities to conduct research in Clinical Microbiology, Molecular Biology, Immunology, Virology, Clinical Biochemistry, etc., in different laboratories (sections) that which are equipped with modern instruments and carried out by expertise in their respective field. Detection of dengue virus serotypes will be carried out at the ICDDR,B by establishing the RT-PCR methods. Facilities for routine microscopic examination, bacteriological culture and selective serology are available at the Shishu hospital and the National Medical College hospital. Facilities will be further improved in all the hospital through the proposed project by supplying required equipment and reagents and test kits and training manpower and offering consultative services.

Data Analysis

Describe plans for data analysis. Indicate whether data will be analyzed by the investigators themselves or by other professionals. Specify what statistical softwares packages will be used and if the study is blinded, when the code will be opened. For clinical trials, indicate if interim data analysis will be required to monitor further progress of the study. (TYPE WITHIN THE PROVIDED SPACE).

Data collection and analysis:

Data will be collected from:

1. Clinical records
2. Laboratory records

Structured questionnaire will be used for collecting data including demographic information (name, age, sex, and address), history of onset of illness, and clinical findings. Laboratory results (CBC, MP, Blood culture etc) and serological findings of each patient will be recorded in a separate form. A designated ID number will identify each study subject. Clinical criteria established by WHO will be used for defining DF, DHF, DSS, and gradation of disease severity.

The investigators, for accuracy, consistency and completeness will review information collected in questionnaires and data forms. Inconsistencies detected will be investigated and clarified. After editing and coding, data will be entered in databases using designed data entry programme. Appropriate range and consistency checks will be built-in. Database will be regularly checked by running frequency distribution and cross tabulations.

Data will be analysed to evaluate: (1) Prevalence rate of dengue infection; (2) rate of primary and secondary dengue infection; (3) distribution virus sero-specific primary and secondary infection and (4) the clinical and epidemiological factors associated with dengue positive cases such as age, gender, time of the year and severity of the disease (DF, DHF and DSS).

Ethical Assurance for Protection of Human Rights

Describe in the space provided the justifications for conducting this research in human subjects. If the study needs observations on sick individuals, provide sufficient reasons for using them. Indicate how subject's rights are protected and if there is any benefit or risk to each subject of the study.

Ethical Implications: The surveillance will identify dengue virus infected patients and subjects who are at risk of DHF and DSS. This will facilitate better treatment response. In this study, the patients will not be exposed to invasive procedure.

Use of Animals

Describe in the space provided the type and species of animal that will be used in the study. Justify with reasons the use of particular animal species in the experiment and the compliance of the animal ethical guidelines for conducting the proposed procedures.

No animals will be used in the proposed study.

Literature Cited

Identify all cited references to published literature in the text by number in parentheses. List all cited references sequentially as they appear in the text. For unpublished references, provide complete information in the text and do not include them in the list of Literature Cited. There is no page limit for this section, however exercise judgment in assessing the "standard" length.

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Dissemination and Use of Findings

Describe explicitly the plans for disseminating the accomplished results. Describe what type of publication is anticipated: working papers, internal (institutional) publication, international publications, international conferences and agencies, workshops etc. Mention if the project is linked to the Government of Bangladesh through a training programme.

Dissemination of the findings of the project will facilitate better preparedness and better management of patients. Technical assistance by the project will contribute to building capacity of the national institutions and also contribute to develop policies by the decision-makers. Mechanism of co-ordination with the government of Bangladesh (GoB) will be developed.

The project members will organise workshops, seminars and meetings with other health care providers and policy makers to share with them with the findings of the project. The project will prepare yearly reports with policy recommendations that will be shared with appropriate staff of the Ministry of Health and Family Welfare (MOHFW). Since the professional from national institutes are also investigators of the project, therefore, findings of the project can easily be disseminated to teachers, students and other professionals. Findings from the study will be published.

Collaborative Arrangements

Describe briefly if this study involves any scientific, administrative, fiscal, or programmatic arrangements with other national or international organizations or individuals. Indicate the nature and extent of collaboration and include a letter of agreement between the applicant or his/her organization and the collaborating organization. (DO NOT EXCEED ONE PAGE)

This is a collaborative study involving ICDDR, B, and Dhaka Shishu Hospital and the National Medical College Hospital, two national institutes that are located in Dhaka city, to collect representative data on viral infection caused by dengue virus serotypes. Within ICDDR,B., there will be collaboration with Public Health Science and Laboratory Science Divisions. The project will also collaborate with the department of Virology, Bangabandhu Sheik Mujib Medical University. The virology department of the medical university will be undertaking the task of setting up dengue virus culture and serological diagnostic methods with assistance from ICDDR, B. In future the department will undertake the role of reference dengue laboratory of the country. The department will also be the resource centre for training of dengue laboratory methods for developing the needed diagnostic capabilities.

Responsibilities of Principal Investigators:

Dr. A Kasem Siddique will have the overall responsibility of the project.
Dr. Mahbubur Rahman will be responsible for laboratory aspect of the project.
Dr. Abdullah H Baqui will be responsible for overlooking the technical aspect of the project.

The PIs will meet at regular intervals for planning and implementation of the project activities.

Responsibilities of Consultants:

The consultants of national collaborating institutes will provide assistance for clinical management of study patients.

Biography of the Investigators

Give biographical data in the following table for key personnel including the Principal Investigator. Use a photocopy of this page for each investigator.

Name	Position	Date of Birth
Dr. A K Siddique	Senior Scientist/Head, ECPP, PHSD ICDDR,B,; Dhaka, Bangladesh	5 December 1938

Academic Qualifications (Begin with baccalaureate or other initial professional education)

Institution and Location	Degree	Year	Field of Study
University of Dhaka, Dhaka, Bangladesh	Bachelor of Medicine and Surgery (MBBS)	1966	Medicine, Surgery, Ob/Gyn
Department of International Health, The John Hopkins University, USA	Master of Public Health	1976	Public health, epidemiology

Research and Professional Experience

Concluding with the present position, list, in chronological order, previous positions held, experience, and honours. Indicate current membership on any professional societies or public committees. List, in, chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. (DO NOT EXCEED TWO PAGES. USE CONTINUATION SHEETS).

Date		Post title and Institution
From	To	
Jun 1985	Current	1. Senior Scientist and Head, Epidemic Control Preparedness Program, Public Health Sciences Division (PHSD) International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). 2. Co-PI of NIH funded study of "Epidemiology and Ecology of *V. cholerae* in Bangladesh". 3. Senior Research Associate, Department of International Health, John Hopkins University.
Jan 1980	June 1984	Consultant Health and Head, State Epidemiology Division, Ministry of Health, Kaduna State, Nigeria.
Jan 1978	Dec 1979	Senior Registrar, Public Health, State Epidemiology Division, Ministry of Health, Kaduna State, Nigeria.
Jan 1977	Dec 1977	Provincial Medical Officer of Health, Zaria Province, Ministry of Health, Kaduna State, Nigeria.
Jun 1976	Dec 1976	Medical Superintendent In-charge, Senior Services Hospital, Ministry of Health, Kaduna State, Nigeria.
Jun 1974	May 1975	Medical Superintendent In-charge, Specialist Hospital, Kafanchan, North Central State, Nigeria.
Mar 1971	May 1974	Medical Officer in-charge, General Hospital, Kafanchan, North Central State, Nigeria.
Feb 1969	Feb 1971	Registrar Surgery, Surgical Unit, Regional Hospital, Mbeya, Tanzania.
Aug 1968	Jan 1969	Registrar Surgery, Mihimbili University, Dar es Salaam, Tanzania.
Jun 1967	Jun 1968	Haematology and Blood Transfusion Unit, Medical College Hospital, Dhaka, Bangladesh.
Sep 1966	Jun 1968	Medical Officer, Emergency & Causality Unit, Medical College Hospital, Dhaka, Bangladesh.

Publications: Selected publications

Faruque SM, **Siddique AK**, Saha MN, Asadulghani, Rahman MM, Zaman K, Albert MJ, Sack DA, Sack RB. Molecular characterization of a new ribotype of Vibrio cholerae O139 Bengal associated with an outbreak of cholera in Bangladesh.J Clin Microbiol. 1999 May;37(5):1313-8.

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Biography of the Investigators

Give biographical data in the following table for key personnel including the Principal Investigator. Use a photocopy of this page for each investigator.

Name	Position	Date of Birth
Dr. Mahbubur Rahman	Associate Scientist ARI Lab, LSD, ICDDR,B	28 January 1953

Academic Qualifications (Begin with baccalaureate or other initial professional education)

Institution and Location	Degree	Year	Field of Study
University of Dhaka, Dhaka, Bangladesh	MBBS	1976	Medicine, Surgery, Ob/Gyn
University of Brussels, Belgium	MS	1991	Molecular biology
University of Brussels, Belgium	PhD	1995	Clinical microbiology

Research and Professional Experience

Concluding with the present position, list, in chronological order, previous positions held, experience, and honours. Indicate current membership on any professional societies or public committees. List, in, chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. (DO NOT EXCEED TWO PAGES. USE CONTINUATION SHEETS).

Date		Post title and Institution
From	To	
Jan 1993	Present	Associate Scientist, ARI Lab, LSD, ICDDRB
1994	1997	Head (Director), Department of Laboratory Services, ICDDR,B
1994	1997	Head, Clinical Microbiology Laboratory, ICDDR,B

Publications: Selected publications

Rahman M and Rahman ATMA. The growing antibiotic resistance: a crisis needs rational use of antibiotics. Orion Med J1998; 1:3-5.

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Rahman M, Mahmood S and Sack DA. Low-cost and early detection of *Shigella dysenteriae* type 1 and *Shigella flexneri* directly from the primary isolation plate by slide coagglutination test (Abstract). Fourth Asian Conference on Diarrhoeal Diseases, Colombo, Sri Lanka, 1987; 75.

Rahman M, Sack DA, Wadood A and Yasmin M. A low-cost and rapid slide coagglutination test for diagnosis of cholera using faecal samples (Abstract). Proceeding of 23d US-Japan Joint Conference on cholera, Virginia, USA, 1987, 88.

Biography of the Investigators

Give biographical data in the following table for key personnel including the Principal Investigator. Use a photocopy of this page for each investigator.

Name	Position	Date of Birth
Dr. Abdullah H Baqui	Adjunct Associate Professor, Dept. of Int'l Health School of Hygiene and Public Health, JHU, MD, USA	31 March 1953

Academic Qualifications (Begin with baccalaureate or other initial professional education)

Institution and Location	Degree	Year	Field of Study
University of Dhaka, Dhaka, Bangladesh	MBBS	1976	Medicine, Surgery, Ob/Gyn
John Hopkins University, USA	MPH	1985	Public health and epidemiology
John Hopkins University, USA	DrPH	1990	Public health and epidemiology

Research and Professional Experience

Concluding with the present position, list, in chronological order, previous positions held, experience, and honours. Indicate current membership on any professional societies or public committees. List, in, chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. (DO NOT EXCEED TWO PAGES. USE CONTINUATION SHEETS).

Date		Post title and Institution
From	To	
Jan 1998	Aug 2000	Senior Epidemiologist and Head, Child Health Programme, PHSD, ICDDR,B
1994	1997	Project Director, MCH-FP Extension Project (Urban), ICDDR,B
1994	1994	Associate Project Director, Urban Health Extension Project, ICDDR,B

Publications: Selected publications

Journal Articles

Baqui AH, Black RE, Arifeen SE, Hill, K, Mitra SN and Sabir AA. Causes of childhood deaths in Bangladesh: results of a nation-wide verbal autopsy study. Bull WHO 1998;76:161-171.

Baqui AH, de Francisco A, Arifeen SE, Siddique AK, Sack RB. Bulging fontanelle after supplementation with 25,000 IU of vitamin A in infancy using immunization contacts. Acta Paediatrica. 1995;85:863-6.

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Baqui AH, Black RE, Sack RB, Chowdhury HR, Yunus M, Siddique AK. Mainutrition, Cell-Mediated immune deficiency and diarrhoea: A community-based longitudinal study in rural Bangladeshi children. Am J Epidemiol 1993; 137(3):355-65.

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Baqui AH, Sack RB, Black RE, Yunus M, Haider K, Alim ARM, Siddique AK. "Enteropathogens associated with Acute and Persistent Diarrhoea in Rural Bangladeshi Children". The Journal of Infectious Disease 1992; 166:792-6

Baqui AH, Sack RB, Black RE et al. Cell-mediate immune deficiency and malnutrition are independent risk factors for persistent diarrhoea in Bangladeshi children. Am J Clin Nutr 1993; 58:453-8.

Baqui AH, Black RE, Sack RB, Chowdhury HR, Yunus M, Siddique AK. Malnutrition, Cell-Mediated immune deficiency and diarrhoea: A community-based longitudinal study in rural Bangladeshi children. Am J Epidemiol 1993; 137(3):355-65.

de Francisco A, Chakrabarty J, Chowdhury HR, Yunus M, Baqui AH, Siddique AK, Sack RB. Acute toxicity of vitamin A given with vaccines in infancy [short report]. Lancet 1993 28;342(8870):526-7

Baqui AH, Arifeen SA, Amin S, Black RE. Levels and Correlates of Maternal Nutritional Status and Consequences for Child Survival in Urban Bangladesh. Eur J Clin Nutr 1994, 48,349-357

Baqui AH, de Francisco A, Arifeen SE, Siddique AK and Sack RB. Bulging fontanelle after supplementation with 25,000 IU vitamin A in infancy using EPI contacts. Acta Paediatr 1995, 84:863-6

Baqui AH, Black RE, Arifeen SE, Hill K, Mitra SN, Sabir AA. Causes of childhood deaths in Bangladesh: results of a nationwide verbal autopsy study. Bull World Health Organ 1998; 76(2):161-71.

APPENDIX

International Centre for Diarrhoeal Disease Research, Bangladesh
Voluntary Consent Form

Title of the Research Project: Surveillance of Dengue viral disease in Bangladesh.

Principal Investigator: AK Siddique, Mahbubur Rahman and Abdullah H Baqui,

Before recruiting into the study, the study subject must be informed about the objectives, procedures, and potential benefits and risks involved in the study. Details of all procedures must be provided including their risks, utility, duration, frequencies, and severity. All questions of the subject must be answered to his/ her satisfaction, indicating that the participation is purely voluntary. For children, consents must be obtained from their parents or legal guardians. The subject must indicate his/ her acceptance of participation by signing or thumb printing on this form.

International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B)
and
Dhaka Shishu Hospital/National Medical College Hospital/Thana
Health Complex, Chattak, Rangamati, Iswarganj and Abhaynagar

Study Title: **Surveillance of Dengue viral disease in Bangladesh.**

CONSENT FORM

(Principal Investigator or Physician of the project will read and explain the consent form to parents or guardian)

You/your patient is possibly suffering from dengue fever and you have come to Dhaka Shishu Hospital/National Medical College Hospital/Thana Health Complex Chattak/Rangamati/Iswarganj/Abhaynagar for treatment. We are conducting a study to know the problems associated with diagnosis of dengue fever. We will treat you/your patient for cure. We request you to help us by allowing you/your patient to participate in our study willingly.

We will collect 5 ml blood once by venipuncture of hand. If necessary a second sample of blood will be collected by same procedure. If required, an X-ray of chest will also be taken. There will be no harm to you/your patient. The results of this test are essential for the treatment. These are all routine and safe investigations. All necessary medical care for symptoms resulting from participation in this study will be provided to the you/your patient without cost. Other than medical care, you/your patient will not receive any compensation for participating in this study. You/your patient will receive appropriate treatment even if he/she does not take part in the study. All information regarding you/your patient will be kept secret.

Physician/investigator and parents/guardian please sign in the form. If parents/guardians are uneducated please provide thumb impression.

Signature of Investigator/ or agents
Date:

Signature of Subject/ Guardian
Date:

APPENDIX

International Centre for Diarrhoeal Disease Research, Bangladesh
Voluntary Consent Form

Title of the Research Project: Surveillance of Dengue viral disease in Bangladesh.

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আন্তর্জাতিক উদরাময় গবেষণা কেন্দ্র, বাংলাদেশ
ঢাকা শিশু হাসপাতাল ও
ন্যাশনাল মেডিকেল কলেজ হাসপাতাল/থানা হেলথ কমপ্লেক্স ছাতক, রাঙ্গামাটি, ইশ্বরগঞ্জ ও অভয়নগর

Study Title: Surveillance of Dengue Viral Disease in Bangladesh

সম্মতি পত্র

(প্রধান গবেষক অথবা গবেষণায় অংশগ্রহণকারী চিকিৎসক রোগী অথবা রোগীর মাতাপিতা অথবা অভিভাবককে এই সম্মতিপত্র পড়ে শুনাবেন এবং ব্যাখ্যা করবেন)

আপনি/আপনার রোগী সম্ভবতঃ ডেঙ্গু জ্বরে আক্রান্ত হয়েছেন এবং এই ঢাকা শিশু হাসপাতাল/ন্যাশন্যাল মেডিকেল কলেজ হাসপাতালে/ছাতক/রাঙ্গামাটি/ইশ্বরগঞ্জ/অভয়নগর থানা হেলথ কমপ্লেক্সে চিকিৎসার জন্য নিয়ে এসেছেন। সুচিকিৎসা দিয়ে আপনাকে/আপনার রোগীকে সুস্থ করে তুলতে আমরা সাহায্য করব। আমাদের দেশের জনগনের ডেঙ্গু জ্বর নির্ণয়ের সমস্যাবলী খুঁজে বের করার জন্য আমরা একটি গবেষণা কার্য চালাচ্ছি। আপনাকে/আপনার রোগীকে নিয়ে এই গবেষণায় যোগ দিতে অনুরোধ করছি।

আমরা আপনার/আপনার রোগীর হাতের সুবিধাজনক যে কোন স্থান থেকে ৫ সি. সি. পরিমান রক্ত পরীক্ষা করার জন্য নেবো। গবেষণার প্রয়োজনে সমপরিমান রক্ত দ্বিতীয়বার নেওয়া হতে পারে। আপনার/আপনার রোগীর বুকের এক্স-রে করারও প্রয়োজন হতে পারে। এতে আপনার/আপনার রোগীর কোন ক্ষতি হবে না। এই পরীক্ষার ফলাফল আপনার/আপনার রোগীর চিকিৎসার জন্য একান্ত প্রয়োজন। এ সবগুলোই নিয়মমাফিক ও নিরাপদমূলক পরীক্ষা। এই গবেষণায় অংশগ্রহণ করার ফলে আপনার/আপনার রোগীর কোন উপসর্গ দেখা দিলে তাকে বিনামূল্যে সব প্রয়োজনীয় চিকিৎসা দেওয়া হবে। চিকিৎসা ছাড়া আপনি আর কোন আর্থিক সুবিধা পাবেন না। আপনি/আপনার রোগী এই গবেষণায় অংশগ্রহণ না করলেও সুচিকিৎসা পাবেন। আপনার/আপনার রোগী বিষয়ক সমস্ত তথ্যাবলী গোপন রাখা হবে।

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

Signature of Investigator/ or agents
Date:

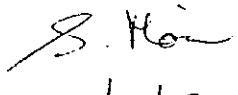
Signature of Subject/ Guardian
Date:

Title: Surveillance of Dengue viral disease in Bangladesh

SUMMARY SHEET OF BUDGET

For 3 years

Items	1st Yr. 2000-2001	2nd Yr. 2001-2002	3rd Yr. 2002-2003	Sub-total
A. FUND ADMINISTERED BY ICDDR,B				
1. Personnel	109,316	146,959	159,467	415,742
2. Consultant (International)	4,000	4,000	4,000	12,000
3. Travel & perdiem	11,334	12,445	11,334	35,113
4. Supplies	2,000	2,000	2,000	6,000
5. Other direct costs	7,110	5,600	5,300	18,010
6. Laboratory investigation	19,749	28,956	17,966	66,671
7. Capital items	46,820			46,820
TOTAL OPERATING COST (ADMINISTERED BY ICDDR,B)	200,329	199,960	200,067	600,356
ADMINISTRATIVE SUPPORT	50,082	49,990	50,017	150,089
TOTAL ICDDR,B ADMINISTERED FUND	250,411	249,950	250,083	750,444
B. SUB-CONTRACT				
1. Utilization of facilities of two general hospitals & assistance to Bangabandhu SM Medical University	6,000	3,000	3,000	12,000
2. Consultant (National)	4,000	4,000	4,000	12,000
TOTAL SUB-CONTRACT	10,000	7,000	7,000	24,000
TOTAL PROJECT COST	260,411	256,950	257,083	774,444



26/4/2000

Surveillance of Dengue viral disease in Bangladesh

Budget Details

for 3 years

A. FUND ADMINISTERED BY ICDDR,B

PERSONNEL

	Pay Level	# of Staff	% of effort	Monthly Rate	1st Yr. 2000-2001	2nd Yr. 2001-2002	3rd Yr. 2002-2003	Sub-total	Total (US $)
Dr A K Siddique*	P-4/8	1	40-60%	9,986	47,933	73,397	77,067	198,397	
Dr.A H Baqui	P-5/7	1	5%	11,225	6,735	7,072	7,425	21,232	
Dr. M Rahman**	NO-C	1	25-80%	1300	3,900	8,190	13,759	25,849	
Physicians Epidemiologist	NO-C	1	100%	953	11,436	12,008	12,608	36,052	
Physicians	NO-A	3	100%	659	23,724	24,910	26,156	74,790	
Research Technician	GS-5	1	100%	362	4,344	4,561	4,789	13,694	
Data Management Officer	GS-5/2	1	100%	380	4,560	4,788	5,027	14,375	
Secretary	GS-5/3	1	100%	398	0	5,015	5,266	10,280	
Driver	GS-2	1	100%	197	2,364	2,482	2,606	7,453	
Messenger/Lab. Attendant	GS-1/1	2	100%	180	4,320	4,536	4,763	13,619	
	TOTAL:				109,316	146,959	159,467		415,742
CONSULTANTS:									
International					4,000	4,000	4,000	12,000	
	TOTAL:				4,000	4,000	4,000		12,000
TRAVEL & PERDIEM									
Local					3,334	4,445	3,334	11,113	
International					5,000	5,000	5,000	15,000	
Perdiem					3,000	3,000	3,000	9,000	
	TOTAL:				11,334	12,445	11,334		35,113
SUPPLIES									
Office supplies					1,000	1,000	1,000	3,000	
Miscellaneous					1,000	1,000	1,000	3,000	
	TOTAL:				2,000	2,000	2,000		6,000
OTHER DIRECT COSTS									
Dissemination workshop						1,800	1,800	3,600	
Training on dengue case management for 2 Project Physicians at Bangkok Children Hospital					4,010			4,010	
Printing					800	1,500	1,200	3,500	
Rent, comm. & Utilities, Shipment of Specimen					800	800	800	2,400	
Repair & maintenance					1,500	1,500	1,500	4,500	
	TOTAL:				7,110	5,600	5,300		18,010
LABORATORY INVESTIGATION COST									
Immunochomatographic tests					4,536	9,072	4,536	18,144	
RT_PCR					3,000	3,000	3,000	9,000	
CBC (TC, DC & HCT, ESR, MP, X-Ray),					6,461	12,921	6,460	25,842	
Lab. Supplies***					5,752	3,963	3,970	13,685	
	TOTAL:				19,749	28,956	17,966		66,671
CAPITAL ITEMS***					46,820	-	-	46,820	46,820
TOTAL OPERATING COST (ADMINISTERED BY ICDDR,B)					**200,329**	**199,960**	**200,067**		**600,356**

* Dr. Siddique: 1st 13 months - 40% and rest of the project duration - 60%

** Dr. M. Rahman: 1st & 2nd Year - 25%, 2nd Year 50%, 3rd Year - 80%

*** Details shown at Page 4

....cont'd

Surveillance of Dengue viral disease in Bangladesh

Budget Details

	1st Yr. 2000-2001	2nd Yr. 2001-2002	3rd Yr. 2002-2003	Sub-total	Total (US $)
B. SUB-CONTRACT					
COSTS FOR UTILIZATION OF FACILITIES OF TWO GENERAL HOSPITALS					
a) Expenses for space, utilities and services	2,000	2,000	2,000	6,000	
b) Furniture	1,500			1,500	
c) Cost for setting virus culture methods at Bangabandhu SM Medical University	2,500	1,000	1,000	4,500	
TOTAL:	6,000	3,000	3,000		12,000
CONSULTANTS:					
National (1 Internal Medicine & 1 Pathology)	4,000	4,000	4,000	12,000	
TOTAL:	4,000	4,000	4,000		12,000
TOTAL SUB-CONTRACT	**10,000**	**7,000**	**7,000**		**24,000**

Surveillance of Dengue viral disease in Bangladesh

Breakdown of Capital items, Lab. investigation and Lab. supply costs:

1. Capital items

Item(s)	Q'tty	Unit Price ($)	Amount ($)
Stethoscope	6	100	600
Computer	1	2,100	2,100
Printer	1	1,000	1,000
UPS	1	240	240
Refrigerator	6	500	3,000
Cold Box	4	50	200
Centrifuge machine	6	2,000	12,000
BP Instrument	6	100	600
Electrophoresis chamber with Power Supply	1	1,400	1,400
Software (statistical package)			1,000
Timer	6	30	180
Incubator	1	3,000	3,000
Liquid nitrogen container	3	1,500	4,500
DNA Sequencer, power supply, gel dryer, film developer, UV light etc.	1	16,000	16,000
Pipettes	4	250	1,000
		TOTAL:	46,820

2. Lab. Investigation

TC, DC, HCT, ESR	1,728	3.43	5,927
MP	1,728	1.68	2,903
Platelet count	1,728	1.22	2,108
X-Ray	432	2.50	1,080
Blood Culture	1,728	8.00	13,824
		TOTAL:	25,842

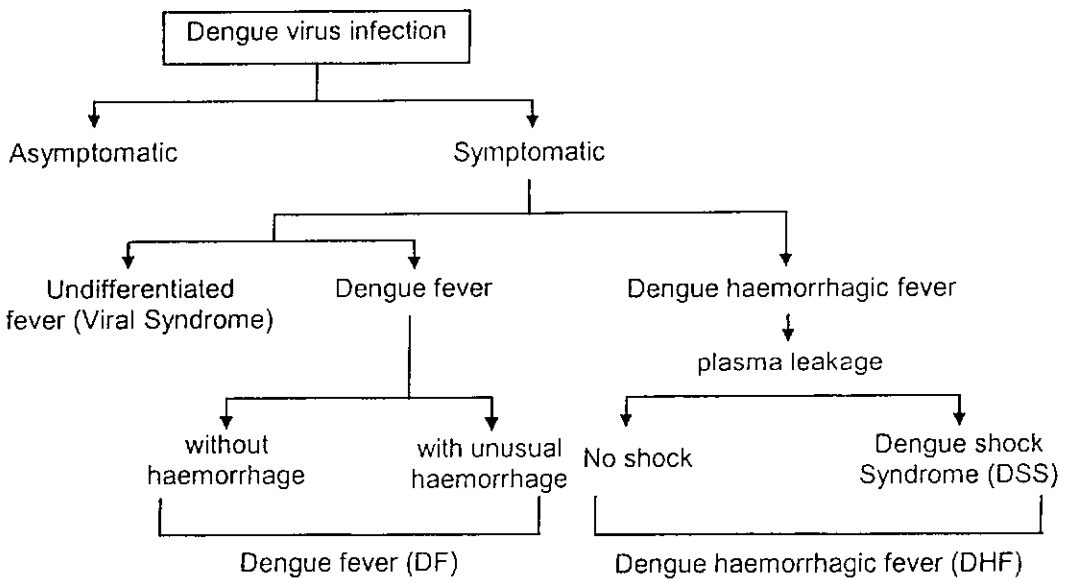
3. Lab supplies

PCR Tube (2000/Pk)	3	175	525
Polaroid Film (667)			350
Agarose	2	370	740
Boric Acid	1	40	40
EDTA	1	170	170
Phenol: chloroform	2	120	240
Trizma base	1	75	75
M. W. Marker (100 bp)			300
Isopropanol (I9516)	2	28	56
Na-acitate	1	35	35
Na-chloride	1	35	35
Freezing vials (500/pk)	10	110	1,100
RNase ZAP (R 2020)			161
Primers			1,000
Probe			500
Ethanol (E- 7023)	10	30	300
Chloroform (C 2432)	6	25	150
Tritox-100	1	55	55
Di-ethyl pyrocabonate	3	33	99
Restriction enzyme			450
Guanidine thiocyanate			205
Mg-chloride	3	33	99
		Sub total:	6,685
Tubes, Glaswares, blood collecting tubes, racks, tips needles, syringe, cotton, bottles, pipettes			7,000
		Sub total:	13,685
		TOTAL:	13,685

Clinical Manifestations of DF and DHF-DSS

The clinical spectrum of dengue infection varies from undifferentiated fever, classical dengue fever syndrome (DF), to severe dengue haemorrhagic fever (DHF) often associated with dengue shock syndrome (DSS). In the young child, dengue infection is usually unrecognisable. However, febrile illnesses occurs with virtually all infections and is often associated with pharyngeal reddening, mild rhinitis, cough, and gastrointestinal symptoms and are diagnosed as pharyngitis, influenza, or upper respiratory infections.

"Spectrum of Dengue Viral Infection"



Dengue Fever (DF)

In the classical case, after an incubation period between 2 to 7 days, there is sudden onset of fever and temperature may rise between 103 to 106°F (39.4 to 41°C). This is usually accompanied by a frontal or retro-orbital headache, backache, pain in muscles and joints. A flushing of the face, puffiness of eyelids, and injection of conjunctival capillaries is often present and is referred as dengue facies. The patient often complains of pain on movement of the eyes. A transient macular, generalised rash that blanches under pressure may be seen during the first 24 h of fever. During the second to sixth day of fever, nausea and vomiting occur. Haemorrhages in the skin (petechiae) are not uncommon. Blood count may show Leucopenia and thrombocytopenia (platelet count below 100,000/mm^3) may be observed. In some cases, DF may be accompanied by bleeding complications, such as bleeding from nose, gums, gastrointestinal bleeding, and haematuria. Within 1 or 2 days after initial remission of fever, a generalised morbilliform rash appears, which spares the palms and soles. It disappears in 1 to 5 days. By the time the rash appears, the body temperature may rise again resulting in the classic biphasic temperature curve.

Dengue Haemorrhagic Fever (DHF)

There are four major clinical manifestations that characterise a typical case of DHF: high fever, haemorrhagic phenomena, and frequently, hepatomagaly and circulatory failure. The distinctive clinical laboratory finding of DHF show moderate to marked thrombocytopenia with concurrent haemoconcentration. The major pathological change that determines the severity of disease in DHF and differentiates it from DF is the leakage of plasma, as manifested by an elevated haematocrit[1] , a serous effusion or hypoproteinamia.

Children with DHF commonly present with a sudden rise in temperature accompanied by facial flush and other non-specific constitutional symptoms resembling DF, such as anorexia, vomiting, headache, and muscle or bone and joint pain. In most cases discrete fine petechiae scattered on the extremities, axillae, face and soft palate, are usually seen during the early febrile phase. In the early febrile phase, the liver is usually palpable and varies in size from just palpable to 2-4 cm below the costal margin and generalized abdominal pain are common. The temperature is usually high (>39 °C) and remain so for 2-7 days. Occasionally, temperature may be as high as 40-41 °C; febrile convulsions may occur, particularly in infants.

The most common haemorrhagic phenomenon is a positive tourniquet test, easy bruising and bleeding and bleeding at venepuncture sites. Present in most cases are discrete fine petechiae scattered on the extremities, axillae, face and soft palate, which are usually seen during the early febrile phase. Epistaxis and gingival bleeding occur infrequently; mild gastrointestinal haemorrhage may be observed during the febrile period.

The liver is usually palpable early in the febrile phase and varies in size from just pulpable to 2-4 cm below the coastal margin. Although liver size is not correlated with disease severity, an enlarged liver is observed more frequently in shock than in non-shock cases. The liver is tender, but jaundice is not usually observed. Spleenomegaly is rarely observed in infants; however, the spleen may be prominent on X-ray examination.

The critical stage of the disease course is reached at the end of the febrile phase. After 2-7 days of fever, a rapid fall in temperature is often accompanied by signs of circulatory disturbance of varying severity. The patient may sweat, be restless, have cool extremities and show some changes in pulse rate and blood pressure. In less severe cases, these changes are minimal and transient, reflecting a mild degree of plasma leakage. Many patients recover spontaneously, or after a short period of fluid and electrolyte therapy. In more sever cases, when plasma loss is critical, shock ensues and can progress rapidly to profound shock and death if not properly treated.

The severity of the disease can be modified by early diagnosis and replacement of plasma loss. Thrombocytopenia and haemoconcentration are usually detectable before the subsidence of fever and the onset of shock.

Dengue shock syndrome

The condition of patients who progress to shock suddenly deteriorates after a fever of 2-7 days' duration. This deterioration occurs at the time of or shortly after, the fall in temperature between the third and the seventh day of the disease. There are the typical signs of circulatory failure: the skin becomes cool, blotchy, and congested; cyanosis is frequently observed; the pulse becomes rapid. Patients may initially be lethargic, then become restless and rapidly enter a critical stage of shock, acute abdominal pain is a frequent complaint shortly before the onset of shock.

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[1] Hematological Values during Infancy and Childhood

Age	Hematocrit (%)		Leucocytes (WBC/mm^3)	
	Mean	Range	Mean	Range
6 mo to 6 yr	37	33-42	10,000	6,000-15,000
7-12 yr	38	34-40	8,000	4,500-13,500

DSS is usually characterised by a rapid, weak pulse with narrowing of the pulse pressure (<20 mm Hg regardless of pressure levels, e.g. 100/90 mm Hg) or hypotension with cold, clammy skin and restlessness. Patients in shock are in danger of dying if appropriate treatment is not promptly administered. Patients may pass into a stage of profound shock, with the blood pressure or pulse becoming imperceptible. However, most patients remain conscious almost to the terminal stage. The duration of shock is short: typically the patient dies within 12-24 hours, or recovers rapidly following appropriate volume-replacement therapy. Pleural effusion and ascites may be detected by physical examination or radiography. Uncorrected shock can give rise to a complicated course, with the development of metabolic acidosis, severe bleeding from the gastrointestinal tract and other organs, and a poor prognosis.

Convalescence in patients with corrected DSS is short and uneventful. Even in cases of profound shock, once shock is overcome, surviving patients recover within 2-3 days, although pleural effusion and ascites may still be present. Good prognostic signs are adequate urine output and the return of appetite.

Common findings during the convalescence of DHF patients are sinus bradycardia or arrhythmia and the characteristic confluent petechial rash with small round areas on normal skin. Maculopapular or rubella-type rashes are less common in DHF than in DF and may be observed either early or late in the disease. The course of DHF is approximately 7-10 days. In general, there is no prolonged fatigue.

Grading of clinical Severity of DHF:

Grade I: Fever accompanied by non-specific constitutional symptoms; the only haemorrhagic manifestation is a positive tourniquet test and/or easy bruising.

Grade II: Spontaneous bleeding in addition to the manifestations of Grade I patients, usually in the forms of skin or other haemorrhages.

Grade III: Circulatory failure manifested by rapid, weak pulse and narrowing of pulse pressure (20 mm Hg or less) or hypotension with the presence of cold, clammy skin and restlessness.

Grade IV: Profound shock with undetectable blood pressure or pulse.

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Management of DF and DHF-DSS

The WHO guideline for the management of DF & DHF-DSS will be followed in the study. Depending upon the severity of infection, three disease entities – DF, DHF and DSS – are recognized. The treatment of each of these is discussed below.

Dengue Fever

The management of DF is symptomatic and supportive.

- Bed rest is advisable during the acute febrile phase.
- Antipyretics or sponging are required to keep the temperature below 40°C. Aspirin should be avoided since it may cause gastritis, bleeding and acidosis; paractamol is preferable.
- Analgesics or mild sedatives may be required for patients with severe pain.
- Oral fluids and electrolyte therapy are recommended for patients with excessive sweating or vomiting.

In DHF-endemic areas, patients should be monitored until after they become afebrile and after platelet counts and haematocrit determinations are normal.

Dengue Haemorrhagic Fever/Dengue Shock Syndrome

General considerations

The major pathophysiologic hallmarks that distinguish DHF/DSS from DF and other diseases are abnormal haemostasis and increased vascular permeability that lead to leakage of plasma. It is thus possible to make early and yet accurate clinical diagnosis of DHF/DSS before the critical stage or before shock occurs, by using the pattern of clinical presentations together with thrombocytopenia and concurrent haemoconcentration, which represent abnormal haemostasis and plasma leakage respectively.

The prognosis of DHF depends on early recognition of plasma leakage. This can be achieved by frequent monitoring for a drop in the platelet count and a rise in the haematocrit level. A rise in haematocrit of 20% or more (e.g. increase from 35% to 42%) reflects a significant plasma loss and indicates the need for intravenous fluid therapy. Early volume replacement of lost plasma with isotonic salt solution can modify the severity of disease and prevent shock.

In mild to moderate cases DHF (Grades I and II), intravenous fluid therapy may be given for a period of 12-24 hours at an outpatient clinic. Patients who continue to have elevated haematocrit, platelet counts below 50,000/mm^3, or present with any type of spontaneous haemorrhage other than petechiae should be hospitalized.

Febrile phase

The management of DHF during the febrile phase is similar to that of DF. Antipyretics may be indicated but salicylates should be avoided. Paracetamol is recommended and should be used only to keep the temperature below 39°C. The following dosages are recommended: under-one year old: 60 mg/dose; 1-2 years old: 60-120 mg/dose; 3-6 years old: 120 mg/dose; and 7-12 years old: 240 mg/dose. Patients with hyperpyrexia are at risk of convulsions.

High fever, anorexia and vomiting lead to thirst and dehydration. Therefore, copiuos amounts of fluids should be given orally, to the extent tolerated. Oral rehydration solutions, such as those used for the treatment of diarrhoeal diseases[1] and/or fruit juices are preferable to plain water. Patients should be closely monitored for the initial signs of shock. The critical period is during the transition from febrile to the afebrile phase, and usually occurs after the third day.

Volume replacement of DHF

Although there is massive plasma leakage, particularly in shock cases, judicious volume replacement is mandatory. The required volume should be charted on a two or three hourly basis or even more frequently in shock cases. The rate of intravenous fluid replacement should be adjusted throughout the 24-48 hour period of leakage by serial haematocrit determinations, with frequent assessment of vital signs and urine output, in order to ensure adequate volume replacement and to avoid over-volume infusion. The volume of fluid replacement should be the minimum that is sufficient to maintain effective circulation during the period of leakage. Excessive volume replacement and continuation after leakage stops will cause massive pleural effusion, ascites, and pulmonary congestion/oedema with respiratory distress when reabsorption of the extravasated plasma occurs in the convalescent stage. In general, the volume required is maintenance plus 5-8% deficit.

Parenteral fluid therapy can be administered in outpatient rehydration units in mild or moderate cases when vomiting produces or threatens to produce dehydration or acidosis or when haemoconcentration is present. The fluid administered to correct dehydration from high fever, anorexia and vomiting is calculated according to the degree of dehydration and electrolyte loss and should have the following composition: 5% glucose in one-half or one-third physiological saline solution (PSS). In the case of acidosis, one-fourth of the total fluids should consist of 0.167 mol/litre of sodium bicarbonate (i.e. three-quarters PSS plus glucose plus one-quarter sodium bicarbonate).

When there is significant haemoconcentration, i.e. haematocrit elevated 20% or more of the baseline value (alternatively, the normal haematocrit value of children in the same age group in the general population may be used to estimate the degree of haemoconcentration), the fluids used for replacement therapy should have a composition similar to plasma. The volume and composition are similar to those used in the treatment of diarrhoea with mild to moderate isotonic dehydration (5-8% deficit).

The necessary volume of replacement fluid is equivalent to the amount of fluids and electrolytes lost: thus, 10ml/kg should be administered for each 1% or normal body weight lost. Maintenance fluid requirements calculated according to the Halliday and Segar[1] formula (Table 1) should be added to the replacement fluid. Since the rate of plasma leakage is not constant (it is more rapid when body temperature drops), the volume and rate of intravenous fluid therapy should be adjusted according to the volume and rate of plasma loss. Plasma loss can be monitored by changes in the haematocrit, vital signs or volume of urine output. However, even where there is massive plasma loss, judicious fluid replacement is necessary to avoid overhydration.

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[1] If the WHO oral rehydration solution (ORS) (90 mmol of Na per litre) is to be used in children under two years of age, additional fruit juice or water should be given in the proportion of one volume of fruit juice (or water) for each two volumes of ORS. The WHO oral rehydration solution consists of: 3.5 g sodium chloride, 2.9 g trisodium citrate dihydrate. 1.5 g potassium chloride, and 20.0 g glucose, dissolved in 1 litre of potable water.

The schedule shown in Table 1 is recommended as a guideline, and has been for moderate dehydration of about 6% deficit (plus maintenance). In older children and adults who weigh more than 40 kgs, the volume needed for 24 hours should be calculated as twice that required for maintenance.

Patients should be hospitalized and treated immediately if there are any of the following signs and symptoms of shock: restlessness/lethargy; cold extremities and circumoral cyanosis; oliguria; rapid and weak pulse; narrowing pulse pressure (20 mm Hg or less) or hypotension, and a sudden rise of haematocrit to a high level or continuously elevated haematocrit levels despite administration of intravenous fluid.

Table 1: Calculations for Maintenance of Intravenous Fluid Infusion*

Body weight (kg)	Maintenance volume (ml) administered over 24 hours
< 10	100/kg
10-20	1000 + 50 for each kg in excess of 10
> 20	1500 + 20 for each kg in excess of 20

* Halliday MA, Segar WE. Maintenance need for water in parenteral fluid therapy. Pediatrics 1957; 19:823

Management of Unusual Manifestations/Complications

Frequent recording of vital signs and haematocrit determinations are important in evaluating treatment results. If the patient presents some indication of secondary shock, vigorous anti-shock therapy should be instituted promptly. These patients should be under constant and careful observation until there is reasonable assurance that the danger has passed. In practice:

- The pulse, blood pressure, respirations and temperature should be recorded every 15 to 30 minutes or more frequently, until the shock has been overcome.
- Haematocrit levels should be determined every two hours during the first six hours, and later every four hours until stable.
- A fluid balance sheet should be kept, recording the type, rate and quantity of fluid administered, in order to determine whether there has been sufficient replacement and correction of fluids and electrolytes. The frequency and volume of urine excreted should also be recorded.

Management of Unusual Manifestations/Complications

The most frequently encountered unusual manifestations are acute hepatic failure and renal failure (which usually follow prolonged shock) that require specific and appropriate treatment. Early blood transfusion in cases of hepatic encephalopathy or Reye's-like syndrome has proved to be life saving in a number of cases, as has haemodialysis in renal failure cases.

Some DHF patients present unusual manifestation with signs and symptoms of CNS involvement, such as convulsion and/or coma. This has generally been shown to be encephalopathy, not encephalitis, which may be a result of intracranial haemorrhage or occlusion associated with DIC. In recent years, however, several cases with CNS infections have been documented by virus isolations from the CSF or brain.

LABORATORY METHODS FOR INVESTIGATION OF DENGUE INFECTION:

Virus Isolation:

Detection of dengue virus by culture is the definitive diagnostic test, but practical considerations limit its use. Most importantly, the period when dengue virus can be successfully detected is brief. Within a day or two after the subsidence of fever, rising levels of antibody interfere with virus culture. Furthermore, dengue virus is generally heat-labile and special precautions must be taken against the thermal inactivation of specimens. Lastly, laboratory facilities to culture viruses are expensive to develop and maintain (WHO 1997). Nevertheless, the most sensitive method of virus detection is inoculation in adult Ades *aegypt* or *Toxorhynchites* species mosquitoes and florescent-antibody staining of mosquito brain tissue with dengue virus-type specific monoclonal antibodies. Mosquito cell line culture (C 6/39 or AP-61 cells) are also being used. This method is less sensitive than inoculation in live mosquito (WHO 1997).

Serological tests:

Serological assays are widely used for laboratory diagnosis of dengue infection. Studies of antibody kinetics showed that in primary dengue infection, IgM antibody to dengue viruses are produced by fifth day of illness and generally persist for 30-60 days, while IgG antibody appears by 14 days of illness and persist for life. In secondary infection, the IgM response is variable, sometime absent, whereas, the IgG antibody rise rapidly 1-2 days after onset of symptoms to reach the levels above those found in primary or past dengue infection. The IgM has been found to be an excellent marker of primary infection while IgG is the preferred marker of secondary infection (Innis 1989; Gubler 1996; Lam 1993). The serological diagnosis of dengue in population exposed to other flaviviruses may result in some degree of uncertainty. Greater confidence in serological diagnosis has been gained by using neutralisation tests. The most commonly used serological test is IgM and IgG antibody-capture ELISAs (Bando 1985; Innis 1989; Kuno 1991). The tests are not suitable for rapid diagnosis or for small number of patients. To facilitate rapid diagnosis, IgM and IgG ELISAs have been modified into immunochromatographic format. The test, which require 7 minutes, antibodies to dengue virus are determined by rapid colloidal gold-based immunochromatographic test for the separate determination of IgM and IgG antibodies in a capture assay format (Vaughn 1998;Sang 1998; Palmer 1999). The IgG cut-off in this test is set to correlate with HAI titre of 1:2560 (the WHO cut-off for secondary dengue infection). The differentiation between primary and secondary infection can be made through a single dilution serum rather than a series of dilution. The test can also be run at the point-of-care and where sophisticated laboratory equipment is not available.

Molecular technique:

Detection of Dengue RNA using dengue–specific oligonucleotide primers, reverse transcriptase and thermostable polymerase in the test "reverse transcriptase-polymerase chain reaction (RT-PCR)" is now being increasingly used (Morita 1991;Lanciotti 1992; Brown 1996; Fang Meiyu 1997; sudiro 1997). Unlike more limited biological amplification through culture, RT-PCR amplification assays can detect a small number of dengue RNA molecules and million-fold enzymatic amplification can be accomplished in a matter of hours.

Collection and handling of specimens

When collecting specimens from patients with suspected dengue infections,

An abbreviated case history, including the following information, should accompany specimens: the patient's name and registration number, address, age, sex, date of onset of illness, date of

hospitalisation, attending physician's name, date of the collection of the specimen, and concise clinical findings should be filled in the prepared form (attached).

Blood may be collected in tubes or vials or on filter paper. High-quality absorbent paper will be used to facilitate the shipment of blood specimens to reference laboratory.

Specimen-collection procedures: tubes or vials

- Aseptically collect 5 ml venous blood
- Use adhesive tape marked with pencil or indelible ink or typewritten or printed self-adhesive label to identify the container. At a minimum, the name of the patient, the identification number and the date of collection should be indicated.
- Use tubes or vials with screw-caps, if possible. Fix the cap with adhesive tape, wax or other sealing material to prevent leakage during transport.
- If a specimen cannot be analysed within 24 hours of being drawn, the serum should be separated from the cells and stores frozen.

Specimen-collection procedure: filter-paper

- With a pencil, write the patient's initials or number on 2 or 3 discs or strips of standardised absorbent paper.
- Collect sufficient fingertip blood (or venous blood in a syringe) on the filter-paper to saturate it through to the reverse side.
- Allow the discs or strips to dry in a place protected from direct sunlight and insects. Preferably, the blood soaked papers should be placed in a stand that allows aeration of both sides. For unusually thick paper, a drying chamber may be useful, e.g. desiccator jar, air-conditioned room, warm-air incubator.
- Place the dried strips in plastic bags and staple them to corresponding laboratory examination request forms. Once dried, the plastic-enclosed strips may be stored at ambient temperature and mailed to laboratory.

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Reviewer - 1

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Donald S. Burke, M.D.
Center Director

October 26, 1999

Professor Barkat-e-Khuda
Division Director
Health & Population Extension Division
International Centre for Diarrhoeal Disease Research, Bangladesh
GPO Box 128, Dhaka-1000
Bangladesh

Dear Professor Barkat-e-Khuda,

Thank you for sending me the research protocol entitled "Surveillance of Dengue Viral Disease in Bangladeshi Children." I apologize for not being able to review it promptly but it arrived shortly before I had other major responsibilities elsewhere. I hope you will find my review useful.

Before I give you my comments I will first give you my refereed opinion scores. They are as follows:

Quality of project – high
Adequacy of Project design – medium
Suitability of methodology - medium
Feasibility within time period – high
Appropriateness of budget – medium
Potential value of field of knowledge – high

Conclusion – I support the application with qualifications on technical grounds. Overall, the research proposal is well thought out and clearly presented. Here are a few suggested changes.

1) Although there is a modest body of literature, including my own presentation, the relationship between climate, the southern oscillation index, and dengue is far from clear. Indeed better surveillance on dengue in several countries could give additional information about the climate drivers of dengue. The third paragraph on page 4 over-emphasizes this issue.

2) I am puzzled why the sample size estimations are powered to detect the percent of febrile children that have dengue rather than estimate the absolute incidence of acute dengue

infections in the population. I am not convinced that it is terribly important to decide if the percent of febrile diseases is 5, 10 or 15 percent; this number will vary substantially according the incidence of other diseases. However, it would be useful to know the absolute impact of dengue in this population because this will drive decisions about resources for prevention and care. It think the sample size estimation calculation should be re-done to power the trial for measurement of the incidence of acute dengue.

3) The case definition for enrollment requires a history of fever for 3-7 days. It is not clear why this duration of fever is necessary for initiation of study. Although it is true that most dengue patients will have disease that lasts this long, by waiting for 3 days of fever before study, many of the viremias will have disappeared, and efforts to isolate and/or amplify dengue viruses from the clinical specimens will suffer. I would suggest that the study enrollment criteria be loosened to include children with 2 days of fever.

4) The section on case management using the guidelines of the World Health Organization is solid. It might be useful for a study nurse and/or a study physician to spend a month during the peak dengue season at a hospital with substantial dengue experience. One excellent hospital is the Bangkok Children's Hospital which has a close relationship with the excellent laboratory at the Armed Forces Research Institute of Medical Sciences in Bangkok. It think it would be an excellent investment to insure the success of the study if a nurse and a physician were to spend 4-6 weeks training in an experienced institution.

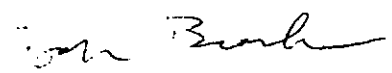
5) On page 11 the word "polymerase" in the title is badly misspelled. The references for methodology are somewhat outdated, and could reflect more recent publication.

6) The affected paragraph in Annex-2 under "Virus Isolation" seems to be out of place. The paragraph deals with IgM serology and should be under the section on the ELISA.

7) The consent form is well constructed. However, it might be useful to explain what dengue fever is in writing because many patients will be confused by the terminology. Also, it would be reasonable to have a witness sign the consent instead of or in addition to the physician investigator.

Overall this is an important study that should generate useful information about dengue and dengue hemorrhagic fever in Bangladesh.

Sincerely,



Donald S. Burke, M.D.
Professor and Director
Center for Immunization Research

Reviewer - 2

Title: Surveillance of Dengue viral disease in Bangladesh children

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		
	Low	Medium	High
Quality of Project			✓
Adequacy of Project Design		✓	✓
Suitability of Methodology			✓
Feasibility within time period			✓
Appropriateness of budget		✓	
Potential value of field of knowledge		✓	

See attached

see attached

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:

Signature: R. Bradley Sack, MD Date: Sept 30, 99

Position: Professor of International Health

Institute: Johns Hopkins Univ.

Detailed Comments:

Please briefly provide your opinions of this proposal, giving special attention to the originality and feasibility of the project, its potential for providing new knowledge and the justification of financial support sought; include suggestions for modifications (scientific or financial) where you feel they are justified.
(Use additional page if necessary)

Title: Surveillance of Dengue viral disease in Bangladesh children

PI: Dr. Kazem Siddiqui

Reviewer: R Bradley Sack

September 30, 1999

The authors propose to study the epidemiology of Dengue Fever in Bangladesh by doing a survey of febrile children in two major hospitals in Dhaka, looking for specific antibodies to the dengue virus. This information would improve the recognition of the disease in Bangladesh, and perhaps in improvement of control measures.

The authors have described the background for the project very comprehensively. The importance of doing this type of a project has been well substantiated.

The project design should be adequate, in that febrile children will be identified in hospital, and blood samples will be taken for antibody determinations.

1) My main question is with the sample size calculations. The prevalence of dengue is expected to be 15% in febrile children, but the total number of children to be sampled is 3,626. I suspect that there is an error in this calculation, and that actual figure may be much less than this. I suggest the authors consult a statistician about this.

If the sample size turns to be much smaller, that means that the budget and time frame for the study can be reduced (unless the authors feel that a 2 ½ year period of surveillance is critical to the study
.)

2) The collaboration with the NAMRU is not identified; personnel and laboratories should be mentioned.

3) No mention of the investigators is made. One needs to know the training of the persons involved to evaluate whether the study can be done. (Some names

are given in the consent form. but nothing is said about the rest of the investigators involved.

4) The budget seems very large, due to the length of the study and the large budget for personnel. No details are given for the capital items, lab costs, travel expenses, or costs to the volunteers (No mention of whether they will be reimbursed is given.) Therefore it is difficult to evaluate it fully.

I have a suggestion. Would it not be useful to do a background survey of antibodies to dengue in different ages of persons, either in the community or in the hospital, to get an idea of its prevalence in the population in general? (from past infection.)

Response to comments by the Reviewers

Review 1

- The reviewer supported the research proposal on the basis of its high quality and potential value of field of knowledge of dengue viral disease. The reviewer mentioned "Overall, the research proposal is well thought out and clearly presented." However, we have following responses to his comments:

1. The third paragraph on page 4 has been modified.
2. Yes, the calculation of sample size estimation is re-done on the basis of the prevalence of dengue in Bangladesh as indicated in study.
3. Yes, the enrollment criteria has been loosened to include children with 2-7 days of fever.
4. Yes, there is scope of clinical training for physician in WHO reference Centre for clinical case management in Bangkok's Children Hospital and laboratory training in Armed Forces Research Institute of Medical Sciences (AFRIMS), Bangkok, Thailand. It is mentioned in the protocol.
5. It has been corrected. AFRIMS uses RT-PCR technique, which was developed by Lanciotti et al in 1992. The technique provides very good and reproducible results.
6. It has been corrected.
7. Yes, the consent form has been modified to explain dengue viral disease.

Review 2

1. Quality of the project is rated to be high.
2. Adequacy is ranked high with Methodology.
3. Sample size: has been modified and the budget has been adjusted accordingly (capital items, lab. Costs, travel exp.)
4. The reviewer also suggested a background summary of antibodies of Dengue in different age groups, either in the community or in hospitals. This will help to get an idea of its prevalence in general.

The protocol was supported with qualification on technical ground as well as on level of financial support.

Surveillance Form for Dengue

1. Date of Examination (Day/Month/Year) /__/__/__/__/__/__/ D D M M Y Y

2. Hospital/Year/Round/Case No /__/__/__/__/__/__/__/ H Y Y R Case
   (Shishu Hosp.=1, National Hosp.=2, Abhaynagar=3, Chattak=4, Iswarganj=5, Rangamati=6)

3. Name of Patient: _____

4. Age: /__/__/__/__/ Y Y M M

5. Sex (Male=1, Female=2) /__/

6. Address: _____

7. Respondent /__/
   (Self=0, mother=1, father=3, brother=3, sister=4, othrs=_____)

8. Religion /__/
   (Muslim=1, Hindu=2,Christian=3, Buddhist=4)

9. Education of the Patient /__/
   (Illeterate=1,Primary=2, School+=3, University=4)

   a) Location of educational institution _____ (specify)

10. Occupation of the Patient _____ (specify)

    a) Location of work place _____ (specify)

11. Occupation of the head of the family _____ (specify)

12. Monthly Income of the head of the family /___/
    (<3000 = 1, 3000-5000 = 2, 6000-10000 = 3, >10000 = 4)

13. Duration of Fever (days) /___/___/

14. Type of Fever (Continuous=1, Intermittent = 2, Biphasic = 3, Others = 4 _____) /__/

15. Taking any medications (no=1, yes=2) /__/

    if yes, Type of medicine (NSAIDS=1, ASA=2, Paracetamol=3) /__/

16. Temperature on admission (Oral/rectal/axillary) /__/__/__/./__/^{0}F

17. Pain symptoms
    a) Headache (absent=1, mild=2, moderate=3, severe=4) /__/
    b) Muscle Pain (absent=1, present=2) /__/
    c) Arthralgia /Bone pain (absent=1, present=2) /__/
    d) Retro-orbital pain (absent=1, present=2) /__/
    e) Abdominal pain (absent=1, present=2) /__/
    f) Others_____(specify) /__/

Case # /__/__/__/

18. Skin manifestations (Absent=1, Present=2) /__/
    If present,
    a) Rash /__/
    (Maculopapular=1, Morbiliform=2, Macular=3)

    b) Location (limbs=1, trunk=2, back=3, Other=4_____) /__/

    c) Pruritis (Absent=1, Present=2) /__/

19. Respiratory Symptoms

    a) Rhinitis (Absent=1, Present=2) /__/

    b) Sorethroat (Absent=1, Present=2) /__/

20. Gastrointestinal Symptoms

    a) Nausea (No=1, Yes=2) /__/

    b) Vomiting (No=1, Yes=2) /__/

    c) Diarrhoea (No=1, Yes=2) /__/

    d) Character of stool (watery=1, bloody=2) /__/

    e) Hepatomegaly (No=1, Yes=2) /__/

    if Yes, _____cm below the RMCL

21. Haemorrhagic manifestations:

    a) Tourniquet test (>20 spots/sq.inch) /__/
    (Positive=1, Negative=2)

    b) Peticheal rash (No=1, Yes=2) /__/

    c) Echymoses/bruise (No=1, Yes=2) /__/

    d) Gum bleeding (No=1, Yes=2) /__/

    e) Nasal bleeding (No=1, Yes=2) /__/

    f) Sub-conjunctival haemorrhage (No=1, Yes=2) /__/

    g) Haematemesis (No=1, Yes=2) /__/

    h) Melaena (No=1, Yes=2) /__/

    I) Blood stool (fresh) (No=1, Yes=2) /__/

    j) Haemoptysis (No=1, Yes=2) /__/

    k) Hamaturia (No=1, Yes=2) /__/

    l) Other _____ /__/

Case # /__/__/__/

22. Plasma leakage (signs/symptoms)
    a) Pleural effusion (Right=1, Left=2, Both=3) /__/
    b) Ascites (Absent=1, Present=2) /__/
    c) Blood Pressure /__/__/__/ mm Hg /__/__/__/ mm Hg
    d) Pulse Pressure /__/__/ mm Hg

23. Other symptoms:
    a) Restlessness (No=1, Yes=2) /__/
    b) Lethargy (No=1, Yes=2) /__/
    c) Cold, Clammy skin (No=1, Yes=2) /__/
    d) Disorientation (No=1, Yes=2) /__/

24. Underlying medical condition _____(specify)

25. Laboratory Findings:
    a) Immunochromatographic rapid test (Negative=1, Positive=2) /__/
       If yes Primary=1, Secodary=2, /__/
    b) Total blood count (WBC _____/mm^3 of blood)
    c) Differential count N =____% E =____% B=____% L =____% M=____%
    d) Total platelet count: _____/mm^3 of blood on _____ day of illness
    d) Haematocrit % _____ on _____ day of illness
    d) SGPT: _____ IU/L
    e) SGOT: _____ IU/L
    f) i) Total Protein: _____gm/l ii) Albumin : _____gm/l
       iii) Globulin : _____gm/l iv) A : G = ___:___
    g) X-ray Chest: Lateral decuibitus (Pleural effusion: Absent=1, Present=2) /___/
    h) Ultrasonogram: Ascites (Absent=1, Present=2) /___/

26. Physician's Signature :_____/___/___/ Date: ____/____/____

27. Supervisor's Signature :_____/___/ Date: ____/____/____

28. DMO's Signature :_____/___/ Date: ____/____/____

Principal Investigator: Last. first. middle

Siddique. AK

Check List

After completing the protocol, please check that the following selected items have been included.

1. Face Sheet Included	X
2. Approval of the Division Director on Face Sheet	X
3. Certification and Signature of PI on Face Sheet, #9 and #10	X
4. Table on Contents	X
5. Project Summary	X
6. Literature Cited	X
7. Biography of Investigators	X
8. Ethical Assurance	X
9. Consent Forms	X
10. Detailed Budget	X

Government of the People's Republic of Bangladesh
Directorate General of Health Services
DGHS, Mohakhali, Dhaka-1212.

No. DGHS/DC/Dengue-1/2000/ 2897 Date: 22.03.2001

To
Director
ICDDR,B
Mohakhali, Dhaka-1212.

Attention: Dr. AK Siddique, Senior Scientist and Head
Epidemic Control Preparedness Programme (ECPP)
ICDDR,B.

Subject: Surveillance activities on Dengue viral disease.

Ref: (1) Your letter dated 14 March 2001.
(2) Your letter dated 21 March 2001.

This is to inform you that we are pleased to accord approval for conducting Sero-epidemiological Surveillance to monitor prevalence and distribution of Dengue Sero-types in two hospitals in Dhaka city and four UZHCs (Chatak, Rangamati, Bajitpur and Abhaynagor). The ethical clearance from ERC of ICDDR,B may be sufficient to address the need for ethical clearance in this matter.

22.3.2001

(Dr. Kanak Ranjan Talukder)
Director (Disease Control)
Directorate General of Health Services
Mohakhali, Dhaka-1212.

CC:
1. Director General of Health Services, DGHS, Mohakhali, Dhaka. (Attention: Asstt. Director, Coordination).
2. Civil Surgeon, Sunamgonj/ Rangamati/ Kishorgonj/ Jessore.
3. Upazilla Health and Family Planning Officer, Chatak, Sunamgonj; Rangamati Sadar, Rangamati; Bajitpur, Kishorgonj; Abhaynagor, Jessore. They are requested to colloberate with the surveillance activities.