RESEARCH PROTOCOL

Protocol No.:

2000 - 38

Project Title: Epidemiology of bronchial asthma among children in rural Bangladesh at Matlab

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: Bronchial asthma, wheeze, Pneumonia, allergen, Bangladesh

Principal Investigator: Takeuchi Haruko K. Zaman   **Division:** PHSD   **Phone:** 8811751/ 2246

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Co-Principal Investigator(s): —

Co-Investigator(s): Md. Yunus, Shams El Arifeen, J. Chakraborty, H.R. Chowdhury, IWATA Tsutomu, WAKAI Susumu

Student Investigator/Intern: —

Collaborating Institute(s): The University of Tokyo

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)
_____

Revised on: 17 October 2000

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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: 1700 children aged 5 years

Total sample size: 1700 ☐

Sub-group _____ ☐ _____ ☐

_____ ☐ _____ ☐

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: _Nissan Scientific funds, Japan_

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?

No

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

Dates of Proposed Period of Support

(Day, Month, Year - DD/MM/YY)

Beginning date _As soon as possible_

End date _6 months from starting_

Cost Required for the Budget Period ($)

a. *Ist Year*   *2nd Year*   *3rd Year*   *Other years*

  44248

b. *Direct Cost :* _35398_   *Total Cost :* _44248_

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.

LA PERSSON

Name of the Division Director

Signature

2/1 2001

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 _K Zaman_

Date: _1 - 1 - 2001_

Name of Contact Person (if applicable)

K. Zaman

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Table of Contents

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 Investigators	TAKEUCHI Haruko and K. Zaman

Project Name	Epidemiology of bronchial asthma among children in rural Bangladesh at Matlab	
Total Budget US $44248	Beginning Date As soon as possible	Ending Date 6 months from starting

Hypothesis

Bronchial asthma causes a great deal of morbidity among children aged 5 years in rural Bangladesh. History of pneumonia in early childhood is associated with allergic sensitization.

Background

Preliminary results suggest that bronchial asthma causes a great deal of morbidity among children in rural Bangladesh. However epidemiological data of bronchial asthma in terms of prevalence, risk factors and its association with host and environmental factors are lacking.

Study Design   Cross-sectional and case control design

Methods

The study will be conducted in rural Bangladesh at Matlab. About 50 villages will be randomly chosen from the intervention area of Matlab Demographic Surveillance System (DSS) area. All children aged 5 years living in those villages (estimated number 1700) will be included in the study. The study will be explained to the caretakers of the children and asked if they agreed to participate. The mothers/caretakers of these children will be asked a questionnaire which include socioeconmic information, any asthmatic attack and any pneumonic episode the child suffered during their infancy (first year of life). Data on pneumonia will be obtained from the record keeping system data of Matlab MCHFP area. Diagnosis of asthma will be done using the adopted questionnaire of International Studies of Asthma and Allergies in Childhood (ISAAC). Children with wheeze at the time of survey (expected number 150) another 150 children matched with age will be referred to Matlab for collection of blood samples. Stool samples and house dust will be collected from these groups of children. Another group comprised of 150 children aged 5 years with history of pneumonia during first year of life and another 150 children without pneumonia matched with age will be randomly selected. Blood samples will be collected from these children as well as house dust from their houses.

Analysis plan

Asthma prevalence, mean environmental mite allergen level, mean environmental endotoxin level and individual total and specific serum IgE will be described with confidence interval. Association between presence of asthma or IgE and history of pneumonia and social and environmental factors will be tested.

Implications of expected results

This study will provide basic epidemiological information of bronchial asthma among children at Matlab, which may be helpful in designing future intervention study.

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

Name	Professional Discipline/ Specialty	Role in the Project
1. TAKEUCHI Haruko	Pediatrician / wheeze control	Principal Investigator
2. K. Zaman	Epidemiologist	Principal Investigator
3. Md Yunus	Senior Scientist and Head Matlab HRP	Co Investigator
4. Shams EL Arifeen	Head and Epidemiologist, CHP	Co Investigator
5. Mr. J. Chakraborty	Senior Manager, CRU, Matlab	Co Investigator
6. H. R. Chowdhury	Senior Physician incharge, Matlab	Co Investigator
7. IWATA Tsutomu	Pediatrician / asthma & allergy	Co Investigator
8. WAKAI Susumu	Neurosurgeon & International health	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. History of pneumonia is positively associated with presence of asthma of children in Bangladesh.

2. Wheezing of children is positively associated with high level of serum IgE, specific IgE RAST score of *D. pteronyssinus* and house dust antigens.

3. History of infant pneumonia has positive association with high serum level of IgE, specific IgE RAST score of *D. pteronyssinus* and house dust antigens.

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 estimate the prevalence of asthma among children aged 5 years in rural Bangladesh at Matlab.

2. To determine risk factors associated with wheezing.

3. To find the association between history of pneumonia or the amount of endotoxin (LPS) in the collected dust of living places, the serum level of IgE, specific IgE RAST score of *D. pteronyssinus* and the presence of parasite infection.

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).**

Epidemiology

In the past 20-30 years there have been many repeated cross-sectional studies which indicate an increased prevalence of allergic respiratory diseases world wide, particularly among children in "western" countries (Burney et al., 1990. Gergen et al., 1988. Manfreda et al., 1993. Anderson et al., 1994). It was shown that there are wide variations exist between countries in prevalence of asthma, its clinical presentation, and natural history (ISAAC, 1998). The differences of the prevalence between countries were 20 to 60-fold and were more within developing countries than in developed countries (Yemaneberhan et al., 1997). Although asthma is a highly hereditary disease, contribution of environmental factors to the increase is speculated. A finding from a study on IgE production of monozygotic twins indicates that the tendency of IgE production is genetically determined and its specificity is governed mainly by environmental influences (Wuthrich et al., 1981). Comparison between former East and West Germany offered a unique opportunity to study the impact of environmental factors on the development of childhood respiratory and allergic disorders in ethnically similar populations. The sensitization to aeroallergens is strikingly more frequent in former West Germany than in former East Germany and this may explain the differences in the prevalence of asthma and hay fever between the two parts of the country (von Mutius et al., 1994).

There are many risk factors of asthma currently confirmed. Risk factors are divided into several categories such as allergic sensitization or exacerbation. These include hereditary atopy, early exposure to protein antigens such as cow's milk or egg white, recurrent respiratory tract infections and indoor and outdoor environmental factors. The most common indoor environmental factor is amount of *Dermatophagoides pteronyssinus* antigen in housedust. The suitable environment for this mite growth is temperature of 20 to 30 degrees C and humidity of 65% to 85% with hiding place such as mattress or mats in closed condition has been regarded as a risk factor. There are reports, however, that endotoxin in housedust plays more important role for severity of asthma in sensitized people than mite allergen (Michel, 1996). Household smoking is another indoor risk factor. Air pollution caused by diesel car

emission gas proved to be a risk factor of allergic sensitization also. On the other hand, a study on early infants showed that presence of endotoxin in housedust prevents their allergic sensitization by enhancing Th1 immunity development (Gereda, 2000). Endotoxin, which is derived from the cell walls of gram-negative bacteria, is a potent inducer of type-1 cytokines interferon gamma and interleukin 12, and can be detected in house-dust in widely varying conditions. In the conditions mentioned above and settings in rural Bangladesh, endotoxin may be abundant in the living places. Besides, expression of atopy is inversely related environmental and household factors such as low socio-economic status, large family size and large number of elder siblings (Strachan, 1985). Fiji Indian children who were in higher socio-economic status compared to Melanesian Fijian children experienced hospital admission from asthma three times more than Melanesian Fijian children. Conversely, admission rates for pneumonia were three times higher for Melanesian Fijians than Fiji Indians. The latter may have more severe asthma and the former had a greater burden of respiratory infection associated with domestic crowding (Flynn, 1994). Current trend of early exposure to protein antigens, increased amount of mite allergens in homes, which have steady suitable environment for mite growth with air conditioners can be said as change to westernized lifestyle or urbanization.

Estimates of current and lifetime prevalence of wheeze and asthma of children in tropical countries in the 1990s showed an increasing trend and found an association between urbanization and prevalence of exercise-induced bronchospasm (Brabin, 1998).

A study in rural Bangladesh using the questionnaire of International Study of Asthma and Allergies in Childhood (ISAAC) showed that prevalence of current wheeze was 9.8% among 6-7 year old children and 7.3% among 13-14 year old children.

Association between bronchial asthma and pneumonia

Respiratory infections have long been recognized as precipitating factors in asthma (Busse, 1993). There have been many reports that history of pneumonia in early life is strongly associated with bronchial asthma. The incidence of wheezing is greatest in the first few years of life (Strachan, 1985), and in children with hereditary atopy/asthma, ALRI caused by respiratory syncital virus (RSV) was associated with increased risk of subsequent allergic sensitization (Sigurs et al., 1995). The study in rural Bangladesh showed that pneumonia was a strong risk factor of current recurrent wheezing among children 6-7 years old and 13-14 years old children with the odds ratio 5.70 [95%CI 3.05-10.65], $p<0.0005$ and 4.00 [2.04-7.84], $p<0.0005$, respectively (Takeuchi, 2000). However, whether this is due

to allergic sensitization is not tested. RSV bronchiolitis during the first year of life apparently is an important risk factor for the development of asthma and sensitization to common allergens during the subsequent 2 years (Martinez et al., 1995; Stein et al., 1999). It was shown in a prospective cohort study that RSV ALRI before 3 years were associated with an increased risk of wheezing during the first 10 years of life. Another report from Germany also showed that repeated episodes of fever and antibiotic treatment in early life were strongly associated with the prevalence of asthma and wheeze at school age.

On the other hand, studies have found that recurrent bacterial infection may prohibit later development of allergy (Holt, 1995; Martinez, 1994). Within asthmatic children the number of fever episodes and antibiotic courses were strongly inversely related to the prevalence of atopy. Asthmatic children with recurrent early childhood infections were at a lower risk of being symptomatic at school age. That means when considering atopic and nonatopic asthmatic children separately, the highest risk of asthma with repeated early childhood infections was found for nonatopic asthma, suggesting that a subgroup of children with a triggering or inducing of asthmatic symptoms through repeated early childhood infections exists within the "asthmatic syndrome" which has a better prognosis and is less related to the atopic phenotype (von Mutis et al., 1999). There were no reports about the outcome when nonatopic wheezing children are left untreated.

Studies on inner-city children in the United States showed that under diagnosis and under treatment were exacerbating factors of asthma (Murray et al., 1997). Patients with ALRI in developing countries are very likely to wheeze and in rural Bangladesh they are prone to develop recurrent wheezing after they recover from it. These children may not be atopic and their recurrent wheezing may clear out by the age of 13. So wheezing should be treated whenever it exists. For this purpose paying attention to slightest wheezing is inevitable.

Since there is a paucity of epidemiological data on bronchial asthma in children in Bangladesh, its research should be given priority. This study will provide basic epidemiological information in terms of prevalence, risk factors and its association with environmental and host factors.

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).

Study settings

The study will be conducted in rural Bangladesh at Matlab, where the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) has been maintaining a field research project since 1963. Matlab is a low-lying riverine area, which lies 45 km south east of Dhaka, the capital of Bangladesh. The principal occupations in the Matlab area are farming and fishing. Since 1966 a Demographic Surveillance System (DSS), which consists of regular cross-sectional censuses and longitudinal registration of vital events, has been maintained in the area (ICDDR,B, 1978). A central treatment facility, staffed by physicians and paramedics provides free therapy for 12,000-15,000 diarrhea patients a year. A Maternal, Child Health & Family Planning Program (MCH-FP) has been in operation for half of the population of the DSS area (current population of DSS is about 210,000) since 1978 and intensive research has been conducted in this population (Bhatia et al., 1980). The other half serves as a comparison area where regular government health care facilities are available. Each community health worker (CHW) in the intervention area covers a population of about 1800. She visits each household monthly and is responsible for distribution of contraceptives to eligible mothers, recording of vital events, and immunization to children, referral of severely sick children and mothers etc. Each CHW in the comparison area covers a population three times larger than a CHW in the intervention area. They are mainly responsible for recording of demographic events.

A community-based longitudinal study on ARI conducted at Matlab showed that the incidence of acute respiratory infections was 5.5 episodes per child per year among children under five years of age (Zaman et

al., 1997). The prevalence of malnutrition is very high among children under 5 years of age in the Matlab area. A previous study (Zaman et al., 1996) revealed that about 73-78% of children were < 2 Z score weight for age, 15-30% were < 2 Z score weight for height and 68-76% were < 2 Z score height for age compared with the NCHS reference population (Hamill et al., 1979). The incidence of diarrhea in a cohort of 705 children aged less than 5 years in the comparison area was 4.6 episodes per year (Baqui et al., 1992).

Study design

Cross sectional and case control study

Study period

The duration of the study will be of 6 months and the data collection will be during the first three months.

Continuation Sheet (Research Design & Methods 2)

Subjects & Sampling

About 50 villages will be randomly chosen from the intervention area of Matlab DSS. All children aged 5 years living in those villages (estimated number 1700) will be included in the study. The study will be explained to the caretakers of the children and asked if they agreed to participate. The mothers/caretakers of these children will be asked a questionnaire which include socioeconomic information and any asthmatic attack. Information about episodes of pneumonia in the first year of life will be taken from the record keeping system (RKS) data. Diagnosis of asthma will be done using the adopted questionnaire of International Studies of Asthma and Allergies in Childhood. (ISAAC). Children with wheeze at the time of survey (cases- expected number 150) another 150 children matched with age will be referred to Matlab for collection of blood samples. These children will be selected randomly from the record of RKS from Matlab MCH FP area. Stool samples and house dust will be collected from these groups of children. Another group comprised of 150 children aged 5 years with history of pneumonia during first year of life and another 150 children without pneumonia matched with age will be randomly selected. Blood samples will be collected from these children as well as house dust from their houses.

Sample size calculation

1. We expect that about 10% children aged 5 years would have wheeze at this population at the time of survey (Takeuchi Haruko, personal communication). To estimate this level of prevalence with ± 2% precision and 95% confidence limit we need a population of 1700. The calculated sample size has been multiplied by 2 to allow stratified analysis.

2. With 80% power at the 5% significance level we need a population of 150 in each group to detect a difference in rates of history of pneumonia of 25% vs 12% between wheezy and non-wheezy children aged 5 years (with 10% loss of follow up).

3. With 80% power and at the 5% significance level we need a population of 150 aged 5 years (with 10% loss to follow up) in each group to detect in prevalence of atopic sensitization of 15% vs 5% between children with or without history of pneumonia during their infancy. Some of the samples for this analysis will be obtained from the above case control design (sample size calculation #2).

Continuation Sheet (Research Design & Methods 3)

Measurement in the field

Questionnaire, interview

The questionnaire items contain questions about wheezing, allergic rhinitis, eczema and risk factors for bronchial asthma. A questionnaire adopted from ISAAC will be used for assessing asthma and allergy prevalence. Risk factors include artificial feeding, introduction of eggs as weaning food, living circumstances such as carpets or cooking apparatus, household smoking and crowdedness, family history, socio-economic status of families and demographic characteristics. It will be translated into Bangla with back translation by some language authority. Answers will be obtained by in-person interview by trained local field workers from guardians mainly mothers, otherwise fathers, grandparents, aunts, sisters or other people who know the child and can answer the questions.

Infancy data

Data on pneumonia and other infectious diseases, such as measles or whooping cough, and records of immunization will be obtained from the records of RKS of Matlab.

Samples collection

Blood and stool samples

Two ml blood and stool samples will be collected from the following groups of children: One hundred fifty children aged 5 years with wheezing and another 150 children without wheezing; another 150 children with history of pneumonia during infancy and another 150 without history of pneumonia. Blood samples will be centrifuged to separate serum. Serum will be kept at −20 degrees C and sent to Tokyo for IgE RAST and IgE RIST test. Serum can be kept at 4 degrees C for the first 2-3 days before frozen. Blood samples will be tested for serum IgE RIST, IgE RAST and stool samples for intestinal parasites.

Continuation Sheet (Research Design & Methods 4)

House dust in the environment

Indoor allergens are leading cause of asthma and other allergic diseases. We want to test the allergen content in dust from homes.

Equipment

A usual vacuum cleaner over 150 W equipped with a paper bag to filter dust will be used for house dust collection (Johanessen, 1998).

Site and time of collection

Sampling site will be the bedding material of the child and the wall of the house at least from 2 sites. Sampling time is 2 min/m^2 for 8 min each site.

Procedure

From the collected dust in the paper bag lighter upper part is removed and heavier dust like part at the bottom of the bag is used for mite allergen test.

Mite-assessment

Mite antigen will be assessed using Akarex test kit.

Endotoxin in the house dust

House dust collection paper is frozen at −20 degrees and carried to Tokyo to test the content of endotoxin in house dust by Limulus test, Limulus Color KY Test Wako (Wako Pure Chemicals Industries Ltd., Osaka, Japan).

Continuation Sheet (Research Design & Methods 5)

Laboratory tests

Serum IgE

Total serum IgE levels will be measured by commercially available ImmunoCAP System IgE FEIA (Fluoroimmunoassay) (Pharmacia K.K., Tokyo) (Zetterstrom et al., 1981, Johanessen, 1988).

Serum specific IgE

Serum IgE antibodies specific to house dust, mite and egg-white will be measured by commercially available ImmunoCAP System RAST (radioallergosorbent test) FEIA (Pharmacia K.K., Tokyo) (Axen et al., 1998; Okundira et al., 1991).

Intestinal parasite infection

About 60 mg of stool samples will be spread and dried on a cellophane paper and tested microscopically for parasites (Ascaris lumbricoides) with Kato-Katz method.

Limulus test

Limulus test uses the characteristics of lysate of hemolymph of horseshoe crab, *Limulus polyphemus* , to form gel-clot by activation of endotoxin.

HS-test Wako is either a quantitative method detecting increment of turbidity due to gel-formation (turbidimetric kinetic assay), with specific computerized equipment, Toxinometer, or a semiquantitative visual gel-clot formation test.

Akarex test

Concentration of mite will be estimated by commercially available kit, Akarex test that determines guanine. There are 3 ways to estimate mite allergen: direct count of mites, semi quantification of mite feces, Enzyme Linked Immuno Sorbant Assay (ELISA) of *Der P.* antigen. Among them ELISA is the most reliable. Because of the difficulty in obtaining laboratory equipment and trained technicians, we will not use this method.

We want to determine guanine, an excretion product of arachnids, in house dust by counting mite fecal pellets as quantitative assay as guanine has been reported to have a good correlation with group I mite allergen assay (Ransom et al., 1991).

A guanine class 0 (<0.6 mg/gm of guanine) of the kit corresponds to a group I allergen content of <2 ug/gm in >80% of the samples, whereas a guanine class 2 or 3 (i.e., >2.5 mg of guanine per gram of dust) corresponds to >10ug/gm of mite group I allergen in >90%of the dust samples.

Advantages of guanine determination include simplicity and economy. Although the quantitative assay for guanine is not generally available, a semiquantitative assay for guanine is commercially

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available and is simple to use and is less expensive than the monoclonal antibody method. This test can be performed easily with minimal training.

Continuation Sheet (Research Design & Methods 6)

Quality control

All questionnaire and data collection instruments will be tested during pretest. The questionnaire will be administered in Bangla. The field supervisor, along with the PIs will be responsible for quality control of data collected through spot-checking and through checking of the completed forms. The supervisor will repeat the questionnaire with some mothers (around 10%), selected at random, on the same day as field worker's interview, and the results will be checked against the field worker's form. There will be regular meetings at Matlab with PIs, supervisors and field workers to resolve any issues.

Data quality of the laboratory procedures will be assured by making appropriate labeling system of samples, preparing proper flow system of the samples, prepare good checking and standardization of the equipment.

Continuation Sheet (Research Design & Methods 7)

Treatment

Children will be given treatment for bronchial asthma and for intestinal parasites.

The children will be referred to Matlab/subcentre and will be examined by the study physician.

The treatment of asthma will be as follows (Mollah et al, 2000):

Mild acute asthma:

Salbutamol inhaler 1-2 puffs every 3-4 hours for 12-24 hours or oral salbutamol 0.2-0.4 mg/kg/day every 8 hourly for 24 hours.

Moderate acute asthma:

Salbutamol inhaler with spacer 2 puffs every 20 minutes for 3 times. If no improvement nebulized salbutamol 0.15 to 0.3 mg/kg. Then to continue salbutamol inhaler 2 puffs 2-4 hourly for 24-36 hours. Oral prednisolone 1-2 mg/kg/day in 3 divided doses for 3 days is to be added.

Severe asthma:

Needs immediate hospitalization. In addition to propped up and oxygen inhalation the patient is to be given nebulized salbutamol 0.15 to 0.3 mg/kg/dose every 20 minutes for 3 times or continuously. Inj. Hydrocortisone 3-4 mg/kg 4-6 hourly or oral prednisolone 2 mg/kg starting dose and then 1 mg/kg 6-12 hourly.

If improvement inhaled salbutamol 2 puffs 2-4 hourly for 3-5 days. Oral prednisolone 1-2 mg/kg/day for 3-10 days.

According to severity of asthma long term management should be given at least for 3 months. The choices of drugs are oral salbutamol, sodium cromoglycate, oral steroid and long acting β_2 agonist (salmeterol).

Treatment of intestinal parasites

Treatment should be given with oral mebendazole 100 mg twice daily for 3 days.

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 large multi-disciplinary international and national scientific research staff. This study will be conducted at rural Matlab. For more than thirty-five years ICDDR,B has been maintaining a field research centre at Matlab. Due to the presence of ongoing health and demographic surveillance system (HDSS), effective referral facilities and well-established infrastructure at Matlab, it offers an excellent research facilities for this study.

Data Analysis

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

Statistical analysis

Data will be analyzed using software package SAS /STATA. Initial exploratory data analysis will be done to find out the distribution of the variables. In cross-sectional analysis dependent variable is the presence of wheeze and the independent variables are individual factors (serum IgE etc.), environmental factors (indoor house dust antigen, endotoxin etc.), social factors and past history of pneumonia. A case control study will be conducted among 150 wheezing cases and 150 controls to find out the factors (indoor house dust, familial history, endotoxin level etc) associated with cases. Multiple logistic regression analysis will be used . The 'logit coefficient' we get is the log of odds ratio (OR). Simply, the OR can be calculated using the corresponding antilog of the logit coefficient.

Proportion of patients with history of pneumonia between wheezy and non-wheezy and between high and low serum IgE will be compared using appropriate tests.

The difference of environmental factors (indoor house dust antigen, endotoxin etc.) between wheezy and non-wheezy groups and between children with high and low serum IgE will be compared.

The atopic sensitization rates will be compared between children with pneumonia and without pneumonia during their infancy using appropriate test (X^2 or Fisher's exact test)

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.

Enrollment of the subjects will be done after signed consent from the mothers/caretakers. They will have the right to withdraw from the study at any time. All collected data will be treated as confidential. No subjects will be deprived of existing care facilities. The study involves no more than minimal risks.

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 use of animals

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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Zaman K, Baqui AH, Yunus M, Sack RB, Bateman OM, Chowdhury HR, Black RE. Acute respiratory infections in children: a community-based longitudinal study in rural Bangladesh. J trop Pediatr 1997; 43: 133-137.

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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.

The findings will be disseminated through presentations in the seminars/conferences. Also the findings will be published in national and international peer reviewed journals.

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)

❑ Department of International Community Health

Faculty of Medicine
Graduate School of International Health
The University of Tokyo

Susumu WAKAI, Professor

He is involved in the fieldwork in developing countries.

❑ Department of Pediatrics

Annex Hospital of Faculty of Medicine
The University of Tokyo

Tsutomu IWATA, Associate Professor

He is involved in the study of asthma.

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. Haruko TAKEUCHI	Visiting Researcher Department of International Community Health Faculty of Medicine Graduate School of International Health The University of Tokyo	4th Aug. 1947

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

Institution and Location Study	Degree	Year	Field of
The University of Tokyo	Medical Doctor	1973	Medicine
Graduate School of International Health The University of Tokyo Health		1998	International

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).

Graduate school student   1998-2000
Department of International Community Health, THE UNIVERSITY OF TOKYO, Tokyo
   General perspectives of International Health

Trainee   Feb 1999
ICDDR,B, Dhaka
   Workshop on emerging and re-emerging diseases

ontinuation Sheet (Research and Professional Experience 2)

Interviewer   Nov 1999
JAPAN INTERNATIONAL COOPERATION AGENCY, The Philippines
   Tuberculosis Control Development and NGO Collaboration

Clinical staff   1983-1998
Department of Pediatrics, TORANOMON HOSPITAL, Tokyo
   Maternal and child health, Treatment of malignant, infectious and allergic diseases

Visiting Researcher   1981-1982
Department of Viral Oncology, UNIVERSITY OF CALIFORNIA, LOS ANGELES, U.S.A.
   Research on prelymphoma cells and onco-virus

Graduate Assistant   1976-1979
Department of Pediatrics, THE UNIVERSITY OF TOKYO, Tokyo
   Treatment of malignant and infectious diseases

Clinical Staff   1975-1976
Department of Pediatrics, KOMAGOME TOKYO METROPOLITAN HOSPITAL, Tokyo
   Treatment of malignant and infectious diseases

Clinical Staff   1974-1975
Department of Pediatrics, TSUKIJI MATERNAL AND CHILD HOSPITAL, Tokyo
   Clinical neonatal medicine, Maternal and child health

Resident   1973-1974
Department of Pediatrics, THE UNIVERSITY OF TOKYO, Tokyo
   Clinical training of general pediatrics

embership

Japan Society of Public Health
Japan Association for International Health
Japan Society of Pediatric Hematology
Societas Pediatrica Japonica

bliography

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Merits and Demerits of Women Doctors. In: Yanagisawa M, editor. *A Practical Guide for Pediatric Training.* Tokyo: Shindan to Chiryou Sha; 1998. p. 146-147.

CV of Dr. K. Zaman

1. **(I) Name** : K. Zaman

   (ii) Designation : Epidemiologist

   (iii) Official address with telephone : Child Health Programme, Public Health
   Sciences Division, ICDDR,B,Dhaka,
   Bangladesh, Tel: 8811751-60 ext. 2246
   Fax: 880 2 8826050
   Email: kzaman@icddrb.org

   (iv) Present residential address : 534/1, Monipur, Mirpur, Dhaka; Tel # 9005841
   with telephone

2. **Academic background:**

Degree	University	Field	Year
PhD	Johns Hopkins University USA	International Health	1999
MPH	Johns Hopkins University USA	International Health	1992
MBBS	Rajshahi University Bangladesh	Medicine, Paediatrics	1978

Field of speciality: Epidemiology, Infectious diseases, International Health, Paediatrics

(a) Research experience: Experienced in the design, implementation, and analysis of data from clinical and community-based epidemiological studies for 20 years
(b) Other experience: Patient care: Clinical care of the patients with diarrhoeal and respiratory diseases
Teaching: Served as a faculty member in different courses on 'Epidemiological methods in Public Health' organized by the ICDDR,B
Teaching Assistant: Department of International Health, Johns Hopkins University, USA
Administration: Overall supervision and management of ICDDRB Matlab Diarrhea Treatment Centre, MCH-FP clinic and Staff clinic

Publications of Dr. K. Zaman

Zaman K. Children's fluid intake during diarrhea: a comparison of questionnaire responses with data from observations. Doctor of Philosophy dissertation. **Johns Hopkins University School of Hygiene and Public Health**, Baltimore, Maryland, USA, 1999.

Zaman K, Baqui AH, Yunus M, Sack RB, Bateman OM, Chowdhury HR, Black RE. Acute respiratory infections in children: a community based longitudinal study in rural Bangladesh. **J Trop Pediatrics** 1997;43:133-137.

3. **Zaman K**, Baqui AH, Yunus M, Sack RB, Chowdhury HR, Black RE. Malnutrition, cell-mediated immune deficiency and acute upper respiratory infections in rural Bangladeshi children. **Acta Paediatrica** 1997; 86: 923-927.

4. **Zaman K**, Zeitlyn S, Chakraborty J, Francisco A de, Yunus M. Acute lower respiratory infections in rural Bangladeshi children: patterns of treatment and identification of barriers. **Southeast Asian J Trop Med Pub Hlth** 1997;28:99-106.

5. **Zaman K**, Baqui AH, Yunus M, Sack RB, Bateman OM, Chowdhury HR, Black RE. Association between nutritional status, cell-mediated immune status and acute lower respiratory infections in Bangladeshi children. **Eur J Clin Nutr** 1996; 50: 309-314.

6. **Zaman K**, Yunus M, Baqui AH, Hossain KMB. Surveillance of Shigellosis in rural Banghladesh : A 10-year review. **J Pak Med Asso** 1991; 41: 75 - 78.

7. **Zaman K**, Yunus M, Hossain KMB. Changing pattern of bio and serotypes of <u>Vibrio cholerae</u> 01 in rural Bangladesh. **J Pak Med Asso** 1987; 37: 57.

8. **Zaman K**, Baqui AH, Yunus M. Hypokalaemia and urinary anomalies in children with diarrhoea in rural Bangladesh. **J Pak Med Asso** 1986; 36: 20 - 21.

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17. Faruque ASG, Rahman ASMM, **Zaman K**. Young childhood diarrhoea management by mothers and village practitioners in rural Bangladesh. **Trop Geo Med** 1985; 37: 223 - 226.

18. Faruque ASG, Rahman ASMM, **Zaman K**. Young childhood diarrhoeal morbidity patterns in rural Bangladesh. **Bang Med J** 1985; 14: 66 - 69.

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20. Baqui AH, Yunus M, **Zaman K**, Mitra AK, Hossain KMB. Surveillance of patients attending a rural diarrhoea treatment centre in Bangladesh. **Trop Geo Med** 1991; 43: 17 - 22.

21. Fauveau V, Yunus M, **Zaman K**, Chakraborty J, Sarder AM. Diarrhoea mortality in rural Bangladeshi children. **J Trop Pediatr** 1991; 37: 31 - 36.

22. Chowdhury HR, Fauveau V, Yunus M, **Zaman K**, Briend A. Is acute watery diarrhoea an important cause of morbidity and mortality among rural Bangladeshi children. **Trans R Soc Trop Med Hyg** 1991; 85: 128 - 130.

23. Baqui AH, Black RE, Mitra AK, Chowdhury HR, **Zaman K**, Fauveau V, Sack RB. Diarrhoeal diseases: The Matlab experience. In: Fauveau V. ed. **Matlab: Women, children and health**. Dhaka: ICDDR,B 1994: 161-186.

24, Chowdhury HR, Yunus M, Khan EH, **Zaman K**, Rahman R. Pivmecillinam resistant Shigella infections in rural Bangladesh. **Trop Doctor** 1995; 25:141-142.

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26. de Francisco A, **Zaman K**, Chowdhury HR, Wahed MA, Chakraborty, Yunus M. Vitamin A toxicity - a case of accidental ingestion. **Trop Doct** 1995; 25: 187.

27. Yunus M, Aziz KMA, **Zaman K**. Message for parents: Diarrhoea. **Child Health Dialogue** 4th Quarter, 1996, 5:5.

28. **Zaman K**, Yunus M, Rahman A, Chowdhury HR, Sack DA. Efficacy of a packaged rice ORS among children with cholera and cholera like illness. **(Submitted)**.

29. **Zaman K**, Sack DA, Chakraborty J, Yunus M, Baqui AH, Black RE. Children's fluid intake during diarrhea: a comparison of questionnaire responses with data from observations. (submitted).

Epidemiology of Bronchiol Asthma in Rural Bangladesh

PI: Dr Takeuchi Haruko & Dr. K. Zaman

	Pay Level	# of Staff	% of effort	monthly rate	months	US $	
SALARY							
Dr. Takeuchi Haruko	1			0	6		
Dr. K. Zaman	1		10%	1382	6	829	
Dr. Md. Yunus	1		5%	1995	6	599	
Dr. Shams El Arifeen	1		5%	1143	6	343	
Mr. J. Chakrabarty	1		5%	1390	6	417	
Dr. H.R. Chowdhury	1		5%	970	6	291	
Field worker		5	100%	210	3	3150	
Medical Officer		1	100%	587	3	1761	
Data Management Asstt.		1	100%	244	2	488	
Community Health Workers		57	5%	170	3	1454	
Field Research Officer		1	100%	364	3	1092	
						10423	10423
TRAVEL COSTS							
local transport						8000	
						8000	8000
SUPPLIES & OTHER COSTS							
Office and field supplies						1000	
Drugs						2000	
Communications, rents and utilities						300	
Printing & publications of forms						2000	
Cold box						200	
Training cost						500	
Generator						500	
Transport to ship specimen						1000	
Service charge						1800	
						9300	9300
INTER-DEPARTMENTAL SERVICES							
lands & water						2500	
Guest house costs						3500	
Medical illustration						100	
Mimeography, Library charge etc.						100	
Stool tests (for parasite)		500		2	1	1000	
Lab cost (serum seperation)		500		0.55		275	
Fuel						200	
						7675	7675

	US $
TOTAL DIRECT COST:	35398
OVERHEAD @ 25%	8850
TOTAL PROJECT COSTS :	44248

Reviewer # 1

Thank you for your E-mail

I read carefully. The study is interesting. In m laboratory a student from your country is working. He is working well (eye infection, adenovirus). I wish he can have an opportunity to study the samples from your country and to apply like this. Please send me application form.

	Rank Score		
	High	Medium	Low
Quality of Project	0		
Adequacy of Project Design	0		
Suitability of Methodology	0		
Feasibility within time period	0		
Potential value of field of knowledge	0		

CONCLUSIONS

1.   support the application:

a) without qualification

b)   with qualification

-    on technical grounds

-    on level of financial support

High

I do not support the application

Detailed comments:

I support the application without qualification. I worry about the budget is enough or not. Now about salary for Dr Takeuchi? How about the cost of laboratory examinations? Small English mistypings were found.

In "Hypothesis", pneumonia is associated with allergic sensitization. Is the plan enough to diagnosis of each pneumonia? Diagnosis of bacteria, virus etc. How about environment such as tubacco etc. food allergy etc?

Title: Epidemiology of bronchial asthma among children in rural Bangladesh at Matlab

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

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

CONCLUSIONS

I support the application:

    a)   without qualification   ✓

    b)   with qualification

        - on technical grounds

  - on level of financial support

I do not support the application

Name of Referee: December 5, 2000

Signature:..................Date: ....

Position: Professor Emeritus, University of Tokyo
                 President Emeritus, National Children's Hospital
Institution:

Detailed Comments

35

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 pages if necessary)

Title: Epidemiology of bronchial asthma among children in rural Bangladesh at
             . Matlab

PI:

Reviewer..Noboru..KOBAYASHI, M.D.
          Professor Emeritus, University of Tokyo
          President Emeritus, National Children's Hospital

In the developed countries including Japan, we are facing problems caused by an increased incidence of bronchial asthma in children. It is considered to be due to the unbalance of Th1 and Th2 cells, that may well be results by a reduction of infectious opportunities for children. Therefore comparative study between the developed countries and the developing country will be essential to solve the problems. The epidemiological study proposed by Haruko TAKEUCHI will throw a light to clarity the relationship between bronchial asthma and infection, eventually establish the prevention of bronchial asthma in children.

Title: Epidemiology of bronchial asthma among children in rural Bangladesh at Matlab

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

Rank Score

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

CONCLUSIONS

I support the application:

       a)   without qualification

       b)   with qualification   ✓

            -   on technical grounds   ✓

   -   on level of financial support

I do <u>not</u> support the application   ✗

Name of Referee:

Signature:....................Date: 10/12/2000

Position: DR. MAHMUDUR RAHMAN
          M.B.B.S (DAC) D P.H (DU)
          Fellow C. Wealth (ENG)

Institution: Prof. of Epidemiology
             NIPSOM. Dhaka.

Detailed Comments

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Review of Research Protocol entitled " Epidemiology of Bronchial Asthma among children in rural Bangladesh at Matlab"

1. The study appears to have 03 components. Cross-sectional (prevalence) and Case-control, with 02 sets of groups. To determine workability or otherwise of this protocol within the stipulated 06 months, it is necessary to know whether the different study designs would be conducted concurrently or in phases.

2. How the 1300 sample children would be selected from the randomly selected 50 villages is not clear. The term "recruited" is used, which indicates purposive sampling. Will this method be appropriate for Cross-sectional Survey? In any case, the methods section on page 3 and Subjects and Sampling on page 13 need to be re-written. The statement "Two groups..........for the study" positioned as it exists is misleading.

On page 13 again in para Nos. 1,2, & 3 respectively, "a population of 1300", "a population of 100" and "a population of 80" is misleading and perhaps need replacement by the word "sample".

3. In the Cross- sectional part of the study data appears to be consisting 5-year recall responses of mothers about asthma and pneumonia episodes of their children. How valid will this data be?

4. In the Case-Control sections, the Case inclusion and exclusion criteria and control of confounders thereof, for the 100 and 80 group children respectively is not spelled out. In the second Case-control group of 80 children how History of Pneumonia can be ascertained? Problems of recall and identification of pneumonia episodes in the past by mothers is not understood. It is not clear whether the costs for testing serum in Tokyo is within or beyond the stated budget.

5. Data Analysis plan regarding Case-control design is not explicit and adequate. Multiple risk factors have been stated to be related to Wheeze and Bronchial Asthma. Does the analysis plan among other things include estimation of Population Attributable Risk for the rural Bangladeshi population? This may be required for recommendations for intervention strategy as distinct from intervention study.

Subject to satisfactory response to above mentioned observations the protocol may be considered for approval.

Reviewer......................

10·12 - 2000

DR. MAHMUDUR RAHMAN
M.B.B.S (DAC) D P.H (DU)
Fellow C. Wealth (ENG)
Prof. of Epidemiology
NIPSOM. Dhaka.

Proposal entitled "Epidemiology of bronchial asthma among children in rural Bangladesh

Responses to External Reviewers' comments

Reviewer 1:

This reviewer supported the application without qualification. The issues and our responses are given below:

Budget of the study:

Yes , we agree with the reviewer that the budget is not enough for the full study. The costs of the laboratory tests (environmental mite allergen and endotoxin tests ~US $ 20,000) which will be done in Japan are not included. Osaka University in Japan and one pharmaceutical company have agreed to provide the amount for the tests.

Salary of Dr. Takeuchi:

The salary of Dr. Takeuchi has not been shown in the budget. It will be provided by the University of Tokyo.

Plan enough to diagnosis each pneumonia?

The study will not perform any microbiological tests to detect causative organisms in pneumonic cases. So it will not be possible to differentiate bacterial and viral pneumonias. We will ask questionnaire to all parents of the study children (about 1300) for any episodes of pneumonia.

How about environment such as tobacco, food allergy etc?

The previous study conducted in Bangladesh in 1999 revealed little or no association between asthma and tobacco and with any particular food (manuscript accepted for publication in the Journal of the Japan Paediatric Society). However, we will include these information (smoking, cooking facilities, food habit etc) in our questionnaire.

Reviewer 2:

This reviewer supported the application without qualification. There were no major comments.

Reviewer 3:

Comment # 1:

A cross-sectional survey of asthma will be conducted among 1300 children aged 10-11 years in the intervention area of Matlab Health and Demographic Surveillance System area. A case control study (expected number 100 cases and 100 controls) will be conducted among children suffering from asthma at the time of survey (cases) and another without asthma matched with age and sex.. The third group comprised of 80 children aged 2 years with history of pneumonia in last one year and another 80 children without pneumonia matched with age selected randomly. The study for the second and third group will be conducted concurrently.

Comment # 2

All children aged 10-11 years in 40 villages will be selected for the study (estimated number 1300). The protocol has been revised accordingly.

Comment # 3

We fully agree with the reviewer's comments about the validity of data with a recall period of five years. However, we believe that the episodes of pneumonia would be very few in this groups of children (10-11 years). We also think that any episode of pneumonia is a serious event for a child. The parents become very concern with it. We will ask the parents about the detailed symptoms and the treatment of severe respiratory infections that their children had during the last five years.

Comment # 4

The cases will be all children with recurrent wheezing at the time of cross sectional survey. They will be included in the study if their parents agree to participate. This has been spelled out in the protocol.

As part of the ARI surveillance study community health workers (CHWs) in the Matlab MCH-FP area detect pneumonia cases in all under five children through monthly home visits. The CHWs have been trained how to diagnose cases of pneumonia according to definition recommended by the WHO (respiratory rate by ages of children, chest indrawing etc). They record all the episode in the record keeping book. The data for the pneumonia cases will be obtained from the book. These cases will be selected randomly.

The costs for testing serum in Tokyo have not been included in the given budget.

Comment # 5

Data analysis section has been elaborated accordingly. To control the effect of confounders, multiple logistic regression analysis will be conducted. From the cross-sectional survey we

will calculate the prevalence rates of bronchial asthma among children aged 10-11 years. We think estimation of population attributable risk from the present design would not appropriate.

Principal Investigator: Last, first, middle _____

International Centre for Diarrhoeal Disease Research, Bangladesh
Voluntary Consent Form
(from cross-sectional survey)

Title of the Research Project: Epidemiology of bronchial asthma among children in rural Bangladesh at Matlab

Principal Investigator: TAKEUCHI Haruko & K. Zaman

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.

Bronchial asthma causes a great deal of morbidity among children in rural Bangladesh. Better understanding of the magnitude of the problem of asthma and methods of prevention are essential for its effective control. We are conducting a study to determine the prevalence, risk factors and its association with environmental and host factors. We are interested to know if any of your children aged five years have any symptoms of Bronchial asthma. If you agree to participate we will ask you some questions regarding the illness of your child and socioeconomic conditions. This will take about 30 minutes to answer the questions. We will refer your child to Matlab for collection of blood samples (2 ml, less than half teaspoonful) and examination of stool samples if your child has any symptoms suggestive of bronchial asthma. A sample of 150 children without any symptoms of asthma will also be referred to Matlab for collection of blood samples and examination of stool samples.

There are minimal risks involved in it. You may decide not to participate in the study at all and this will not affect your child's treatment. You are at liberty to withdraw your child from the study at anytime without any obligations and jeopardizing medical care and treatment.

If you allow your child to participate in the study, please sign your name or give left thumb impression below.

Consent: The study described above has been explained to me and I voluntarily consent to allow my child in this study

Signature of Interviewer
Date:

Signature of Guardian
Date:

International Centre for Diarrhoeal Disease Research, Bangladesh
Voluntary Consent Form
(Children for atopic sensitization)

Title of the Research Project: Epidemiology of bronchial asthma among children in rural Bangladesh at Matlab

Principal Investigator: TAKEUCHI Haruko & K. Zaman

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.

Bronchial asthma causes a great deal of morbidity among children in rural Bangladesh. Better understanding of the magnitude of the problem of asthma and methods of prevention are essential for its effective control. We are conducting a study to determine the prevalence, risk factors and its association with environmental and host factors. One of your child suffered from pneumonia during their infancy (under one year of age). We are interested to know if your child has allergic sensitization to some antigens common to asthma patients. If you agree to participate we will ask you some questions regarding the illness of your child and socioeconomic conditions. This will take about 30 minutes to answer the questions. We will refer your child to Matlab for collection of blood samples (2 ml, less than half teaspoonful). A sample of 150 children without any history of pneumonia during their infancy will also be referred to Matlab for collection of blood.

There are minimal risks involved in it. You may decide not to participate in the study at all and this will not affect your child's treatment. You are at liberty to withdraw your child from the study at anytime without any obligations and jeopardizing medical care and treatment.

If you allow your child to participate in the study, please sign your name or give left thumb impression below.

Consent: The study described above has been explained to me and I voluntarily consent to allow my child in this study

Signature of Interviewer
Date:

Signature of Guardian
Date: