

Date 3/5/98

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator Dr. A.H. Baqui/ICDDR,B
Dr. R.E. Black/JHU

Trainee Investigator (if any) _____

Application No. 98-010

Supporting Agency (if Non-ICDDR,B) USAID/Washington & JHU/Maryland, USA

Title of Study A community-based, randomized

Project status:

- controlled trial to assess the effect of () New Study
inc supplementation in <5 year old () Continuation with change
Bangladeshi children during diarrhoea on () No change (do not fill out rest of form)
clinical course of diarr, subsequent diarr,
and ARI morbidity, and growth

Circle the appropriate answer to each of the following (If Not Applicable write NA).

1. Source of Population:
(a) Ill subjects Yes No
(b) Non-ill subjects Yes No
(c) Minors or persons under guardianship Yes No

2. Does the study involve:
(a) Physical risks to the subjects Yes No
(b) Social Risks Yes No
(c) Psychological risks to subjects Yes No
(d) Discomfort to subjects Yes No
(e) Invasion of privacy Yes No
(f) Disclosure of information damaging to subject or others Yes No

3. Does the study involve:
(a) Use of records, (hospital, medical, death, birth or other) Yes No
(b) Use of fetal tissue or abortus Yes No
(c) Use of organs or body fluids Yes No

4. Are subjects clearly informed about:
(a) Nature and purposes of study Yes No
(b) Procedures to be followed including alternatives used Yes No
(c) Physical risks Yes No N/A
(d) Sensitive questions Yes No N/A
(e) Benefits to be derived Yes No
(f) Right to refuse to participate or to withdraw from study Yes No
(g) Confidential handling of data Yes No
(h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure Yes No N/A

5. Will signed consent form be required:

- (a) From subjects Yes No
(b) From parent or guardian (if subjects are minors) Yes No

6. Will precautions be taken to protect anonymity of subjects Yes No

7. Check documents being submitted herewith to Committee:

- Umbrella proposal - Initially submit an overview (all other requirements will be submitted with individual studies).
✓ Protocol (Required)
✓ Abstract Summary (Required)
✓ Statement given or read to subjects on nature of study, risks, types of questions to be asked, and right to refuse to participate or withdraw (Required)
✓ Informed consent form for subjects
✓ Informed consent form for parent or guardian
✓ Procedure for maintaining confidentiality
— Questionnaire or interview schedule *

* If the final instrument is not completed prior to review, the following information should be included in the abstract summary:

1. A description of the areas to be covered in the questionnaire or interview which could be considered either sensitive or which would constitute an invasion of privacy.
2. Examples of the type of specific questions to be asked in the sensitive areas.
3. An indication as to when the questionnaire will be presented to the Cttee. for review.

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

Malyri

RESEARCH PROTOCOL

1. Title of Project: A community-based, randomized, controlled trial to assess the effect of zinc supplementation in <5 year old Bangladeshi children during diarrhoea on the clinical course of diarrhoea, subsequent diarrhoea and ARI morbidity, and growth

2. Investigators :

2a. Name of the Principal Investigator
including position and qualifications:

Dr. Abdullah H Baqui, MBBS, MPH, DrPH
Senior Epidemiologist and Head, Child Health Program
Public Health Sciences Division ICDDR,B

Dr. Robert E Black
Professor and Chairman
Department of International Health
Johns Hopkins University School of Hygiene and Public Health
Baltimore, Maryland, USA

2b. Co-Investigators: Professor Patrick Vaughan, PHSD, ICDDR,B
Dr. Md. Yunus, PHSD, ICDDR,B
Dr. Shams El Arifeen, PHSD, ICDDR,B
Mr. J Chakraborty, PHSD, ICDDR,B
Dr. Van Ginneken, PHSD, ICDDR,B
Dr. George Fuchs, CSD, ICDDR,B

3. Name of the Division/ Branch / Programme of ICDDR,B under which the study will be carried out:

Child Health Programme
Public Health Sciences Division

4. Dates of Proposed Period of Support
(Day, Month, Year - DD/MM/YY) :

01/06/98 to 31/07/99

5. Cost Required for the Budget Period:

5a. Direct Cost : US\$ 154,791

Total Cost : US\$ 193,489

6. Sponsor(s): Johns Hopkins University and USAID/Washington

7. 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.

J Patrick Vaughan

Name of the Division Director

J Patrick Vaughan

Signature

(Act Div. Dir.)

25 April 1998

Date of Approval

8. Certification by the Principal Investigator

(PHS ID)

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.

9. Signature of PI

Marley D.

Date:

29-4-98

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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.

Zinc deficiency is highly prevalent in developing country children, including in Bangladesh. Recent controlled trials have shown the therapeutic effects of zinc supplementation on the duration and severity of acute and persistent diarrhoeal episodes. Controlled trials have also documented the preventive effects of zinc supplementation on the incidence of acute and persistent diarrhoea, dysentery, and acute lower respiratory infection (ALRI). Zinc has been shown to improve growth and immune status of supplemented children. Diarrhoea affects zinc status by increased intestinal losses, impaired absorption and less dietary intake. During diarrhoea a higher zinc requirement to maintain a positive zinc balance has been documented. Thus, in developing country children, such as those in Bangladesh, where diarrhoea rates are high and dietary zinc insufficiency is highly prevalent, episodes of diarrhoea may result in further deterioration of zinc status which in turn may lead to increased incidence of diarrhoea, more severe and longer duration diarrhoea, increased incidence of ALRI and retarded growth.

While the importance of zinc supplementation and maintenance of zinc-sufficient status have been established, how this can be achieved in a program situation is not clear. Unlike vitamin A, the total available body store of zinc is small and maintenance of a zinc-sufficient status requires regular intake. Roy et al. in Bangladesh documented some reduction of morbidity for 2-3 months period following zinc supplementation during and shortly after diarrhoea. This study had a small sample and only one episode of diarrhoea was treated with zinc.

This study is based on the premise that if all episodes of diarrhoea are treated with zinc, the severity and duration of the treated episodes will be reduced. In addition, a positive zinc balance will be maintained in most children which in turn will reduce morbidity and improve growth. A community-based, prospective, randomized, controlled trial is proposed to assess the effect of two weeks of zinc therapy for all episodes of diarrhoea in children during a year follow-up on the clinical course of diarrhoea, subsequent diarrhoea and ALRI morbidity and growth. If the proposed intervention is proved to be successful, it can be easily incorporated with the existing diarrhoeal disease control program, which should significantly improve child health and survival.

DESCRIPTION OF THE RESEARCH PROJECT

Hypothesis to be tested:

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

In an under-five year old population where zinc deficiency is common, zinc supplementation given as a therapy for two weeks in addition to oral rehydration therapy (ORT) during diarrhoeal episodes will:

- 1) Reduce the duration and severity of the treated diarrhoeal episode;
- 2) Reduce subsequent diarrhoeal illness;
- 3) Reduce diarrhoea episodes related hospitalization;
- 4) Reduce subsequent acute lower respiratory infections (ALRIs); and
- 5) Improve growth of supplemented children.

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**).

Primary Aims:

To evaluate the effect of two weeks of zinc therapy in children for all episodes of diarrhoea during a year follow-up on:

- 1) the clinical course of the treated episodes of diarrhoea, including the severity, duration and hospitalization rates;
- 2) subsequent morbidities due to diarrhoea and acute respiratory tract infections; and
- 3) subsequent growth in weight and height of children.

Secondary Aims:

- 1) Evaluate the effect of zinc supplementation on use of oral rehydration therapy (ORT) and use of other drugs for the treatment of the diarrhoea
- 2) Evaluate the effect of zinc supplementation on childhood mortality

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).

Childhood Morbidity and Mortality:

Every year, about 12 million children in developing countries die before reaching their fifth birthday, many during the first year of life. Over 70% of these deaths are due to diarrhoea, acute lower respiratory infections (ALRIs), measles, malaria or malnutrition, and often to a combination of these conditions. It has been estimated that, in developing countries, diarrhoea and ALRIs each account for an estimated 19%, and measles accounts for another 7% of all childhood deaths (Murray CJL and Lopez AD, 1996). Fifty four percent of the deaths are associated with malnutrition (Pelletier DL et al, 1993). In addition, at least three out of every four episodes of childhood illnesses are caused by one or more of these five conditions (Murray CJL and Lopez AD, 1996).

In the past decade, major progress has been made to reduce childhood morbidity and mortality through universal childhood immunization, control of diarrhoeal diseases and acute respiratory infections, nutrition programmes and through implementation of other primary health care activities. Despite this progress, mortality and morbidity rates are still unacceptably high, particularly in South Asia and Sub-Saharan Africa.

A recently conducted nation-wide verbal autopsy study in Bangladesh found that acute lower respiratory infections (ALRIs) are the most important killer of Bangladeshi children (Baqui et al, in press). About one out of every four under-five deaths were associated with a confirmed diagnosis of ALRI and about two out of every five infant deaths were associated with a confirmed diagnosis of ALRI. Diarrhoea was associated with 19% of all child deaths -- about 13% had confirmed symptoms of diarrhoea. Of the later, about half had either persistent diarrhoea or dysentery. Furthermore, about half of the diarrhoea-associated deaths had concomitant malnutrition. The findings of this study were consistent with the overall developing country estimates and three other studies conducted in nationally representative samples in Bangladesh (Kamal et al 1993, Petra Osiniski [personal communication], Salway and Nasim 1994).

Childhood Diarrhoea:

Diarrhoeal diseases remains one of the major causes of morbidity and mortality in the developing world. Annually, the developing country children suffer from more than one billion diarrhoeal illness episodes (Snyder JD, Merson MH, 1982) and these episodes account for an estimated 2.5 million infant and child deaths (Murray CJL and Lopez AD, 1996).

Young children in developing countries suffer from up to 10 episodes of diarrhoea per child per year. Some of these children suffer from diarrhoea for 50 to 60 days in a year (Black et al., 1982; Mata, 1978; Leeuwenburg et al., 1978; Guerrant et al., 1983; UNICEF, 1985). Diarrhoea is the most important infectious cause of malnutrition in children of the developing countries. It has been estimated that 20-25 percent growth differential between the developed and the developing countries is due to infectious diarrhoea (Guerrant et al. 1986a). Some have argued that the effect of an annual occurrence of 3 to 10 diarrhoeal episodes per child in the most critical developmental age of life may have an even greater impact on the society than the staggering mortality (Guerrant et al. 1986a).

Although studies suggest that certain children may be genetically more susceptible to diarrhoea (Rutter et al. 1975; Levine et al. 1979a), the principal risk factors associated with mortality and severe morbidity due to diarrhoea are all related to poverty. Two-thirds of the world's population lives in developing countries characterized by extreme poverty, lack of adequate food, high illiteracy, intense overcrowding in substandard housing, grossly inadequate or almost non-existent potable water supply and sanitation, improper food handling and storage, poor personal hygiene, lack of access to health care etc. All these factors interact to produce the 'diarrhoea-malnutrition-diarrhoea' chain.

At the turn of the century, conditions of under-development existed in many parts of the now-developed world with high mortality and morbidity due to diarrhoeal and other infectious diseases. The precipitous decline in mortality and morbidity that these countries enjoyed over the next 30 to 50 years was the result of rapid socioeconomic development, which enabled large scale capital expenditure for safe water supply and sewage disposal, improved housing, education, improved weaning foods etc. and occurred in the absence of specific knowledge of the etiology and therapy (Levine et al. 1979b; Newsholme, 1935). This fact has persuaded some to argue that the overall socioeconomic development leading to better nourished, better cared-for children are more important than specific scientific advances in diagnosis and therapy.

While doubtless that overall socioeconomic development is the ultimate goal, unfortunately, it seems extremely unlikely that such development will occur soon in many of the developing countries. Therefore, alternative approaches are required to reduce the diarrhoea-associated morbidity and mortality. What is needed are simple, practical and relatively inexpensive technologies that would do the most good for the greatest number and at the lowest cost. Oral rehydration therapy (ORT) is a unique example.

The vast majority of the diarrhoeal illnesses are acute, short-lived and until recently the majority of the diarrhoeal deaths were due to acute diarrhoeal illnesses with concomitant dehydration, acidosis and/or renal failure. The discovery of oral rehydration therapy (ORT), a simple yet highly effective and inexpensive technology has largely simplified the treatment of acute diarrhoea and resulted in an unquestionable decline in diarrhoeal mortality and hospitalization (Rahman et al. 1979; Pierce, 1977; Pizzaro, 1988; UNICEF, 1987).

Despite its great benefits, ORT alone does not seem to be the ultimate answer for all types of diarrhoea. Effective ORT programs have been shown to reduce diarrhoeal mortality by 50 percent but the rate of diarrhoea-associated mortality remains relatively high. This indicates that mechanisms in addition to dehydration are important causes of mortality. Persistent diarrhoea

along with its consequent deterioration of nutritional status, is perhaps the most significant (Guerrant et al. 1986a). Persistent diarrhoea has been shown to be responsible for a substantial portion of diarrhoea-associated and overall mortality in young children in many of the developing countries (Fauveau et al. 1994, Guerrant et al. 1986a; Bhan et al. 1986; WHO memo. 1988).

Diarrhoea and Nutrition:

The classic work by Scrimshaw et al. (1968) postulated that malnutrition and infection, including diarrhoea, have a bi-directional causal relationship wherein malnutrition predisposes to diarrhoea and conversely, diarrhoea exerts a negative impact on nutritional status. Afterwards, many studies have shown that diarrhoea leads to malnutrition (Rowland et al. 1977; Martorell et al. 1975; Black et al. 1984a). Some researchers have found that nutritional status is strongly predictive of the prevalence of diarrhoeal illnesses (Delgado et al. 1983; James, 1972; Tomkins, 1981; Trowbridge et al. 1981; Black et al. 1984b). Among the studies measuring the incidence rates, those of Tomkins (1981), Delgado et al. (1983), Sepulveda et al. (1988) and El Samani et al. (1988) found nutritional status to be predictive of the frequency of the diarrhoeal episodes. In contrast, Black et al. (1984b), Chen et al. (1981) and Bairagi (1987) reported from Bangladesh that malnutrition was a determining factor in diarrhoeal duration leading to a higher prevalence, but it did not increase the overall incidence. Subsequent studies at Matlab, Bangladesh documented that malnutrition and cell-mediated immune deficiency are independent risk factors for acute and persistent diarrhoea (Baqui et al., 1992; Baqui et al 1993).

The higher incidence of persistent diarrhoea in malnourished children possibly occurs due to changes in certain host factors. Severely malnourished children have been shown to have defects in cell-mediated immune function (Schlesinger et al. 1974; Purtilo et al. 1976) and a decrease in IgA containing cells in the jejunal mucosa (Green & Heyworth, 1980). The immunosuppressive effects of undernutrition together with observations that the infection is of greater severity, lasts longer and recurs more frequently in malnourished children, are well established (Chandra, 1978; Chandra, 1983).

Nutrition, Immunity and Diarrhoea

Until recently, only extreme undernutrition - Kwashiorkor and marasmus -- were thought to decrease the immune capacity of the host to levels of clinical importance. However, recent studies suggested that even sub-clinical malnutrition can decrease the immune capacity of the host. Of special significance are the observations from Bangladesh and Peru that the child's ability to respond to standard skin test antigens (cell-mediated immune status) was a significant predictor of the risk of developing diarrhoea (Black et al., 1989; Baqui et al., 1993). Compared to immunocompetent children, the Bangladesh study observed a 40% excess diarrhoea and about 100% excess persistent diarrhoea among anergic children. Zaman et al (1996) observed similar increased risk of ARI in Bangladeshi anergic children. This effect of impaired cellular immunity persisted after adjusting for the effects of age and nutritional status (as assessed by anthropometry).

The above studies could not assess the factors responsible for the impaired cell-mediated immunity, but it has been shown in other populations that cell-mediated immune deficiency in apparently well nourished children can occur following a variety of viral and bacterial infections

(Kauffman et al 1976) as well as due to single nutrient deficiency, such as in vitamin A, pyridoxine, folic acid, iron and zinc deficiency (Beisel 1982).

With the increasing recognition of the critical interaction between malnutrition, immunity, and infection, efforts are now focussed in the development of nutritional approaches to child survival. The wide-spread cell-mediated immune deficiency in otherwise well nourished (by anthropometry) children and its independent effect on the increased incidence of infectious diseases, such as diarrhoea and ARI has led to investigate the role of single nutrients, such as zinc, on cell-mediated immunity.

Prevalence of Zinc Deficiency:

Zinc deficiency is highly prevalent in developing country children including in Bangladeshi children with rates as high as 38-50% (Sarker SA et al, 1985; Sachdev et al., 1988; Sazawal S et al., 1995). Data from studies carried out at ICDDR, Bangladesh suggest that at least one-third of the Bangladeshi children are zinc deficient defined by serum zinc levels below 10.0 $\mu\text{mol/l}$ (Sarker SA et al, 1985; MA Wahed, personal communication).

Zinc, Infection, and Immunity:

There is increasing evidence from recent controlled trials of the potential role of zinc in the prevention and treatment of diarrhoea, malaria, and pneumonia, and its role in the improvements of growth and immune status.

A number of recent studies documented a significant preventive effect of zinc supplementation on diarrhoeal morbidity. A community-based, double blind, randomized trial in India observed 26% lower diarrhoeal incidence and a 35% lower prevalence in children aged >11 months who received daily zinc supplementation for a six months period (Sazawal et al, 1997). A trial of zinc supplementation (10mg/day) in growth retarded Vietnamese children observed a 71% lower diarrhoeal incidence (Ninh NX, 1996). A similar trial in Mexico found a 36% lower incidence of diarrhoeal episodes in zinc supplemented (20mg/day) children (Rosado J et al, 1997). Sazawal et al (1996) also observed that zinc supplementation reduces the incidence of persistent diarrhoea in zinc deficient children and reduces the risk of dysentery in boys. A report from Guatemala also indicated that zinc supplementation reduces the incidence of all diarrhoea (Ruel et al. 1997).

A number of recent trials have documented the therapeutic effect of zinc in diarrhoea. Sazawal et al. (1995) examined the effect of zinc supplementation (20 mg elemental zinc/day) in children aged 6 to 35 months with acute diarrhoea in a community-based, double blind, controlled trial. Zinc supplementation was associated with significant overall reduction of 23% in the risk of continued diarrhoea on a given day and a 37% reduction in the severity of diarrhoeal episodes when zinc was provided early in the episode. When supplementation was initiated within 3 days of the onset of diarrhoea, there was a 39% reduction in the frequency of episodes lasting more than 7 days after the beginning of the treatment. Similar reduction in diarrhoeal episode duration and severity were observed with zinc supplementation during acute diarrhoea in Bangladesh (Roy SK 1997) and during acute and persistent diarrhoea in hospitalized children in India (Sachdev et al. 1988, Sachdev et al 1990).

The link between diarrhoea and zinc is well established. Diarrhoea leads to zinc loss and abnormalities of zinc metabolism. Substantial amount of zinc is lost during acute diarrhoea. It was observed that daily losses of zinc in children with diarrhoea could be as high as 159 µg/kg/day, compared with 47 µg/kg/day in control children (Castillo-Duran et al, 1988).

The possible mechanisms for the effect of zinc supplementation on the duration and severity of diarrhoea include improved absorption of water and electrolytes by the intestines (Ghishan FK 1984, Patrick J et al 1980, Golden BE et al 1985, Patrick J 1978), regeneration of gut epithelium (Bettger WJ et al 1981, Elems ME and Jones JG 1980, Arcasoy A 1990, Moran JR 1985, Weaver LT et al 1985, Roy SK et al 1992), increased levels of enterocyte brush-border enzymes (Gebhard RL et al 1983, Jones PE et al 1981), and enhanced immunologic mechanisms for the clearance of infection. Zinc supplementation improves immunity (Fraker et al. 1979; Fraker JP 1983; Sazawal et al. 1997) and thereby may promote rapid clearance of diarrhoeal pathogens from the intestine. Clearance of intestinal parasites, such as *Strongyloides ratti* in zinc deficient rats occurred significantly earlier and more efficiently after zinc supplementation (Fenwick PK et al. 1990). It is also possible that improved appetite and dietary intake result in shorter duration of diarrhoea (Brown KH 1994).

Although oral rehydration solutions treat dehydration efficiently, the need for a symptomatic treatment of diarrhoea still persists (Bull WHO 1994;72:945-55). A large proportion of diarrhoeal episodes are currently treated with antibiotics and other drugs (Baqui et al. 1993). Use of antibiotics/drugs for most episodes of diarrhoea are not justified. The wide-spread use of antibiotics for the treatment of diarrhoea is not just an inappropriate use of scarce health resources, it can contribute to the emergence of multiple-drug resistant strains. As zinc reduces the severity and duration of all types of diarrhoea, it is proposed as a new candidate with possible anti-diarrhoeal activity. Zinc supplementation during diarrhoea should also improve growth and reduce subsequent diarrhoea and ARI morbidity.

Zinc and ARI

The literature on the association of zinc and respiratory morbidity is limited. Several studies reported a higher ARI morbidity in zinc deficient children (Lombeck I et al 1988, Van Wuiwe JP et al 1986, Bondestam M et al 1985). The results of zinc supplementation trials on ARI morbidity are mixed; two studies reported a reduction in respiratory morbidity (Sempertegui F et al 1996, Ninh NX et al 1996) while two others did not (Rosado JL et al 1997, Ruel MT et al 1997). A recent community-based controlled trial conducted in India documented a substantial efficacy of zinc supplementation in reducing pneumonia morbidity. Zinc supplementation was associated with 56% reduction of the incidence of pneumonia and 51% reduction in the days spent with pneumonia (Sazawal et al. Submitted for publication). A possible mechanism for the effect of zinc supplementation on reduction of pneumonia morbidity is enhanced immune status. Another possible mechanism of zinc supplementation is a direct antiviral effect (Eby GA et al 1984, Al-Nakib W et al 1987).

Zinc and Growth

In Bangladesh, a majority of the children are either undernourished or marginally nourished (Baqui et al, 1993). Protein-calorie undernutrition is intimately associated with micronutrient deficiencies (Khanum et al. 1988). Conversely, zinc supplementation has been shown to improve

growth, particularly in low birth weight infants (Walravens PA et al 1983). The catch-up growth may be due to improved appetite (Brown KH 1994), better absorption of nutrients (Ghishan FK 1984, Patrick J et al 1980, Golden BE et al 1985, Patrick J 1978) and increased protein synthesis after zinc supplementation (Khanum et al. 1988).

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 Design:

A community-based, prospective, randomized, controlled trial is proposed to assess the effect of two weeks of zinc therapy in children during diarrhoea on the clinical course of diarrhoea, subsequent diarrhoea and ARI morbidity and growth. Villages in the Matlab field area of ICDDR,B will be randomly assigned to one of the two groups, zinc plus ORT or ORT only. Both groups will also receive the usual diarrhoea care available in the community. Attempts will be made to keep the baseline differences to a minimum so that any differences in the outcome variables can be interpreted as treatment effects. Baseline differences, if any will be adjusted using appropriate statistical methods during data analysis.

Study Population:

The trial will be carried out at the Matlab field research area of ICDDR,B, a rural, riverine sub-district of Bangladesh where diarrhoea-related activities have been conducted since 1963. Matlab is located about 55 km south-east of Dhaka, the capital of Bangladesh. The field area was originally developed for field evaluation of cholera vaccines. To support field studies, a Demographic Surveillance System (DSS) was established in 1966. Over the years, the system has been refined and is currently operational in 144 villages containing about 210,000 population. The DSS gathers vital event information, such as births, deaths, migrations on a regular basis through home visits. The demographic database is computerized and updated regularly. In late 1977, ICDDR,B started implementing a comprehensive maternal child health and family planning (MCH-FP) program in half of the DSS villages. The remaining half has continued to receive health and family planning services from the Government of Bangladesh and serves as comparison area for the MCH-FP program area.

Since the inception of research work in the Matlab area, diarrhoeal patients have been treated by ICDDR,B in a diarrhoea treatment centre (DTC) located at Matlab town. In addition to Matlab DTC, three community-operated diarrhoea treatment centres (COTCs) established between 1978

and 1982 also provide treatment to diarrhoea patients. In late 1980's ICDDR,B started an ARI program in Matlab and opened an ARI ward to treat severe ALRI cases. Oral rehydration therapy (ORT) and referral services for diarrhoea are provided in the community by a cadre of community health workers (CHW -- one per two thousand population in the MCH-FP area and one per four thousand population in the comparison area) and by 'Bari Mothers' (these are community volunteers who serve as depot-holders of ORS packet, distribute ORS and provide advice to diarrhoea patients -- there is one for about 50 persons). The CHWs also provide treatment and referral services for ARI. The CHWs visit each household once in a month and during home visits collect some selected information, including information on diarrhoea and ARI morbidity. This data collection system, known as Record Keeping System (RKS), is computerized. These resources provided the opportunity to conduct numerous clinical and epidemiological studies on diarrhoeal diseases, which have provided important insights into the epidemiology, etiology, and therapy of all types of diarrhoea.

Matlab is fairly representative of most parts of rural Bangladesh. In 1995, the crude birth rates in the MCH-FP and comparison areas were 25.2 and 27.8 per 1,000 populations respectively. The crude deaths rates and infant mortality rates in the MCH-FP and comparisons areas respectively were 7.3 and 8.4 per 1000 populations and 51.1 and 78.6 per 1,000 live births. Although there has been a decline in diarrhoeal death rates over time, the rates are still high. In 1995, diarrhoea accounted for about 12% of infants deaths and about a third of all deaths in children 1-4 years of age. When diarrhoeal deaths are dis-aggregated into deaths due to acute watery diarrhoea, dysentery or persistent diarrhoea, it becomes evident that persistent diarrhoea and dysentery were more important causes of deaths than acute watery diarrhoea in children over one year of age. This findings suggest that the strategy for diarrhoeal control programs needs to be broadened to include nutritional interventions to prevent or manage persistent diarrhoea.

In 1996, Matlab treated about 12,000 diarrhoea patients. 2,387 of them were from the DSS area which translates into a hospital admission rate for diarrhoea of 11.4/1,000 population. The diarrhoea related hospitalization rate in <5 children was much higher (60/1,000 or 6%). There were considerable variations in hospitalization rates between villages and this was primarily a function of distance from the DTC.

Selection of Study Villages and Randomization:

About 40 DSS villages around Matlab will be selected for the study to ensure adequate sample size. The selected villages will be ranked using hospital visit incidence rates. To minimize baseline differences in diarrhoea incidence and diarrhoea related hospitalization rates, one village from each pair of villages will be randomly assigned to treatment (zinc plus ORT) and the other in the comparison group (ORT).

Subject Selection:

Children from the selected villages meeting the following inclusion and exclusion criteria will be enrolled in the study:

Inclusion criteria:

- a) Children below 5 years of age at enrollment;
- b) All new born babies who will be born in the study villages over the study year will be included in the study to ensure adequate infants in the trial;
- c) Permanent resident of the selected villages

Exclusion criteria:

- a) severely malnourished children requiring hospitalization

Verbal informed consent will be obtained. As this is a community treatment trial of a new therapy, consent will be obtained from the communities (villages) through meetings where the purpose of the study including potential benefits of the new therapy will be presented. The study will involve some additional data collection from participating households and <5 children in those households. Consent for this component of the study will be obtained from parents of all selected children.

Table 1. Main outcome variables, incidence/prevalence/proportion in the control group, expected reduction in the treatment group and data collection methods

Outcome Variables	Incidence/prevalence/proportion in the control group	Incidence/prevalence/proportion in the treatment group (Expected reduction)	Sample size required in each group (after making allowances for 10% drop outs)	Data collection method
<u>Therapeutic Effects on Diarrhoea</u>				
Episode duration of non-dysenteric diarrhoea (mean±sd)	7 (±2) days	6.3 (±2) (10% reduction)	141 episodes	2-monthly home visits
Episode duration of dysentery (mean±sd)	10.7 (±2)	9.63±2 (10% reduction)	63 episodes	2-monthly home visits
Percent of episodes lasting more than 7 days	25%	20% (20%) reduction)	1,246 episodes	2-monthly home visits

Outcome Variables	Incidence/prevalence/proportion in the control group	Incidence/prevalence/proportion in the treatment group (Expected reduction)	Sample size required in each group (after making allowances for 10% drop outs)	Data collection method
<u>Preventive effect on Diarrhoea</u> Percent days with diarrhoea	12%	9.6% (20% reduction)	1277* child-weeks observed	2-monthly home visits
<u>Combined therapeutic and preventive effect on diarrhoea</u> Reduced diarrhoea related hospitalization rate	6/100 child years observed	4/100 child years observed (33% reduction)	2,158 child years observed	Passive surveillance at Matlab DTC
<u>Preventive effects on Pneumonia</u> Percent of children with pneumonia over the study year	16%	12.8% (20% reduction)	920* child week observed	2-monthly home visits
Reduced pneumonia hospitalization rate	8%	5.6% (30% reduction)	1989 child years observed	Passive surveillance at Matlab ARI ward
<u>Effect on Growth</u> Effect upon weight gain (mean±sd)	-2.0 ±1.0 z-score weight for height	-1.5±1.0 z-score weight for height	150	Weight and height of the 6-11 month old cohort (n=430) will be measured at enrollment and thereafter every 2 month
Effect upon linear growth (mean±sd)	-2.0 ±1.0 z-score weight for height	-1.5±1.0 z-score weight for height	150	
Post-neonatal mortality	2.5%	1.25% (50% reduction)	2,005 child-years observed	DSS data
<u>Other diarrhoea episode related outcome</u> Percent received ORT	50%	70%	114 episodes	2-monthly home visits
Percent received other drugs	20%	10%	647 episodes	

* Sample size adjusted assuming a design effect of 3

Sample Size Estimation:

When interventions are allocated to communities (in this cases villages) but individuals within the communities are subject of interventions, sample size determination can be done in two ways. One option is to consider communities as unit of observation. However, for calculating the required number of communities, the community level incidence rates (or proportions or means) and variance estimates are required and such data are often not available. Alternatively, sample size can be calculated for individuals but then the sample size needs to be multiplied by the appropriate 'design effect'. The design effect will be 1 if there is no heterogeneity between communities in the outcomes of interest, in the sense that the variation between the community-specific rates or means is no more than would be expected to occur by chance due to sampling variations. For most outcomes, however, there will be real differences between communities (i.e., the design effect will be >1), and in these circumstances the required study size will be greater than with individual allocation.

Sample sizes were calculated to detect assumed differences between the treatment and comparison groups (based on literature) with 80% power and 95% significance level. As we calculated sample sizes for individuals, we assumed a design effect of 3 for most outcomes. For some outcome variables (e.g., hospitalization rate), the sample is child period observed and for some others (e.g., duration of diarrhoea), the sample is number of episodes. When the sample is number of episodes, the sample sizes have been increased by 10% to account for drop outs. When the sample sizes are child periods observed, about 10% more children will be enrolled in the study to ensure that an adequate number of child periods are observed.

Diarrhoea related hospital admission rates of Matlab DSS population for the years 1995 and 1996 were used to estimate the expected hospital admission rate in the comparison population. With the assumptions that annual incidence of admissions for diarrhoea in children <5 years of age in the control population will be 6%, in the treatment population it will be reduced to 4.0% (a 33% reduction), and for a Type I error=0.05 and Type II error=0.2, a sample size of 1,962 child-years observed per group will be required. The sample size requirement for other hypothesized changes are smaller.

For example, assuming that 20% of the episodes in the comparison area will receive drugs and zinc supplementation will reduce this by 50%, the sample size requirement will be 647 episodes in each group.

Allowing for drop outs etc., to detect the assumed reductions in the diarrhoea and ALRI-related hospitalization rates about 2,158 children aged less than five years will be enrolled in each group for a year. For outcomes, e.g., incidence and prevalence of diarrhoea and ALRI, severity and duration of diarrhoea, acceptance of ORT, use of other drugs, information from each group will be collected by 2-monthly home visits.

Description of the Intervention:

'Bari mothers' (Community Volunteers or CVs) serve as depot holders of ORS packets. They distribute ORS packets and provide education on how to prepare and administer ORS to

diarrhoea patients. They also keep simple records of patients treated such as the DSS registration numbers of the patients and number of ORS packets given to each patient.

At the beginning of the study, the CVs in the treatment villages will be requested to serve as depot holders of zinc syrup as well. As there is an inherent demand for drugs for diarrhoea in the population, both the CVs and the study population should welcome this arrangement. The CHWs and CVs in the treatment villages will be trained on how to use zinc syrup as a therapy during diarrhoeal episodes. They will then treat diarrhoeal episodes in children with ORT and zinc. The children in comparison villages will continue to receive ORT. The therapy will begin as soon as the episode is reported to a CHW or CV. During each episode, the study children in the intervention villages will receive 20 mg of elemental zinc per day for 14 days regardless of the duration of diarrhoea. In addition, the study children in both the treatment and control villages will continue to receive usual feeding advice and any other treatment they normally seek. Attempts will be made to the extent possible through the ongoing program of ICDDR,B to maximize the use of the study recommended therapy. However, no attempt will be made to change the usual care-seeking pattern of the study population.

The CVs in the treatment villages will record the number of zinc bottles given to patients in addition to the numbers of ORS packets and their DSS registration numbers. To estimate the proportion of diarrhoeal episodes treated with ORT or Zinc plus ORT, data from the CVs records will be compared with the RKS data of number of episodes.

Data on various study outcomes and compliance with zinc therapy will be collected by study workers who will not be involved in the implementation of the intervention.

Data Collection

Baseline Enrollment:

Matlab DSS assigns unique identification numbers to households and individuals who are permanent residents of the area. A list of all <5 children residing in the study villages including their household identification numbers will be obtained from the DSS database. The study will use the DSS registration numbers which will allow linking with other data sets, such as DSS and RKS.

The baseline enrolment will include obtaining informed consent of the parents of the study children, retrospective morbidity data for one month, feeding information, and anthropometric measurements. The anthropometry will be conducted only in the 6-11 month old cohort. In addition, information on household composition and socioeconomic conditions will be obtained from the DSS database by linking with this database. Baseline data will be used: a) to determine the child's eligibility in the trial, b) to describe the population, c) to determine baseline differences between the treatment and control groups, and (d) to adjust for baseline differences, if any during data analysis.

Data Collection to Ascertain Various Outcomes:

The data collection methods to be used and the sample size to be followed will vary for different outcomes. These have been summarized in table 1. The study will have dedicated staff to collect the necessary data so that the data collection is independent of the implementation of the intervention. However, for some outcome, the DSS (e.g., mortality) and RKS (e.g., pneumonia) data will be used. The RKS data is collected by CHWs who are also providers of services in these communities. The validity of this providers' records (RKS data) will be determined by regularly comparing these data with the data collected by independent study staff. This should help ensure the quality of the RKS data. The RKS will provide data from much larger observation periods for the study children. However, for most outcomes, the data collected by the study staff will be adequate. The following sections describe the various data collection methods and data sources.

Data on Diarrhoea and Pneumonia Related Hospitalizations:

For these particular outcomes, data from all 4,316 study children will be required. However, no active surveillance will be set up. Data on hospital visits by the study children will be collected from Matlab DTC, ARI ward and the COTCs. The study population used these centres almost exclusively for diarrhoea-related hospitalizations. Some children with pneumonia may also go to the government Thana Health Centre. Thus, this centre will also be included in the pneumonia surveillance. Use of hospital records of these Centres should provide almost complete information on these outcomes. The under-estimation, if any should be minimum and unbiased. Data on duration, type, and severity of diarrhoea or pneumonia, presence of any other concurrent illness, weight, height, and information on treatment sought before hospitalization including use of zinc and/or ORT will be collected.

Data to Assess the Therapeutic Effect on Diarrhoeal Episodes and for Diarrhoea and ARI Incidence and Prevalence:

To assess the therapeutic effect of zinc supplementation on diarrhoeal episodes and on subsequent diarrhoea and ARI prevalence, data will be collected from the study children by trained and dedicated community workers (CWs) through periodic home visits. To avoid the effect of seasonality, about equal numbers of children from the treatment and control villages will be visited in each week. These CWs will have no involvement with the implementation of the intervention. They will visit each study child in their homes once every two months and will collect data on diarrhoea and pneumonia in the last week, including severity and duration. They will also collect data on use of treatments for current or recent episodes. The recall period will be seven days except for episode duration and use of treatments to minimize recall lapses. This household visit will provide data from 25,896 child-weeks observed and will be adequate for all analysis except hospitalization due to diarrhoea and pneumonia and mortality.

Data on compliance with zinc intake, adverse events (if any) related to zinc supplementation and use of other drugs

Data related to these outcomes will be collected by CWs during their 2-monthly household visits. During each visit the CWs will record information on number of stools, consistency of stools, vomiting, fever, blood in the stool, ORS intake, zinc intake in the treatment villages, other types of care sought including visit to a physician or hospital or admission to a hospital for diarrhoea.

To assess compliance of zinc therapy, the CWs will collect detailed information on when the diarrhoeal episode started, when the zinc therapy started, and how regularly the syrup was given. The mothers will be requested to show the bottle containing the zinc syrup so that the accuracy of the reporting regarding zinc therapy can be ascertained and the amount of zinc intake can be estimated.

Data on dietary intake of zinc

Dietary data will be collected from a sample of children to assess the dietary intake of zinc which would help identify any differences in zinc intake in the treatment and control children and would help with external validity of the study.

Individual dietary data will be collected for a 10% systematic sample of the study children of both the treatment and control villages at baseline and thereafter once every two months. Trained field interviewers will ask each mother of the sampled children to report all foods eaten by her child in the 24 hours preceding the interview. Using pre-measured utensils and spoons, the interviewers will determine and record the types and amount of all foods that were consumed during the previous day. Nutrient contents including zinc content of the foods consumed will be calculated using food composition tables relevant for Bangladesh (Nutrient contents of Indian food. C. Gopalan, National Institute of Nutrition, Hyderabad, India).

Estimation of serum zinc level

Blood will be collected from a sample of the following five groups of study children for estimation of serum zinc level:

- i) Children with acute diarrhoea and from the treatment villages;
- ii) Same children 3 weeks after recovery;
- iii) Age and gender matched children with acute diarrhoea and from the control villages;
- iv) Same children 3 weeks after recovery; and
- v) Age and gender matched healthy control children.

An earlier ICDDR,B study (Sarker et al., 1985) found mean ($\pm$ sd) serum zinc level of 11.0 ($\pm$ 2.0) μ mol/l in healthy children and 8.3 ($\pm$ 2.2) μ mol/l in diarrhoeal children during acute phase. To detect this difference 11 children in each group are needed. To detect the difference observed by Roy et al., (1997) in the mean serum zinc levels of children who received zinc supplementation for two weeks and who did not, 33 children are required in each group. The study proposes to measure the serum zinc level of 50 children of each type or a total of 250 children. First, 50 children with acute diarrhoea from the treatment villages (index children) will be randomly selected over the study year. An age and gender matched child with acute diarrhoea from a control village and an age and gender matched healthy child for each index child will be selected within one week of selection of the index child. About 3 ml blood will be collected from each of the selected children. All blood samples will be collected in plastic containers carefully washed to make them zinc free. Serum zinc will be measured using an atomic absorption spectrophotometer.

Anthropometry

The 6-11 months old cohort of the study children (n=430) will be included in this component of the study because this age group is expected to benefit most in terms of improvements in weight gain and linear growth from zinc supplementation. The sample size will allow stratified analysis by initial nutritional status of the child. The cohort will be followed for a year by trained health assistants (HAs). Measurements will include weight, length, and mid-upper arm circumference (MUAC) at enrollment and thereafter every other month. Naked weight will be obtained to the nearest 50 grams on a Salter scale standardized with a one kg weight prior to each weight measurement. Recumbent lengths will be measured to the nearest 0.1 cm with a locally constructed length board with a footplate and a head bar. MUAC will be measured to the nearest millimeter by TALC insertion tapes. The mean of two consecutive measurements will be recorded as the observed value. To ensure reliability and accuracy of the anthropometric data, WHO measurement and standardization protocols will be followed. The anthropometric data will be compared to the standards according to the NCHS reference data and the nutritional status assessed by Z scores (Waterlow JH, 1972).

Effect on mortality:

To examine any possible mortality differentials between the treatment and comparison groups, the mortality data, particularly of the post-neonatal period collected by the DSS will be used. The sample size is unlikely to be adequate to show any significant group difference in mortality. If there is any trend, the analysis will be repeated at the end of two years using two years of mortality data.

Methods to Ensure Data Quality:

To ensure data quality, the study supervisors and investigators will make spot checks. In addition, a 5% sample of study children will be re-interviewed and re-measured within two days of the original interview/measurement.

Data Management:

All questionnaires and data forms will be reviewed by the investigators for accuracy, consistency and completeness. Whenever necessary, the CWs/HAs will make additional field visits to clarify inconsistencies or collect missing information. After editing, the data will be entered in databases using on-line custom-designed data entry programs. Necessary range and consistency checks will be in-built. Data will be periodically checked by running and reviewing frequency distributions and cross-tabulations.

Data Analysis:

Baseline characteristics of the treatment and control groups will be examined for group comparability. Any significant baseline differences will be controlled for during data analysis.

The frequency distribution will be examined to assess the distribution of data. If the data is not normally distributed, decisions about need for data transformation and on appropriateness of

statistical tests will be made. Appropriate statistical methods will be used to account for the correlated nature of the data.

A number of different analytical strategies will be used to assess the effect of zinc supplementation on various outcomes.

Therapeutic Effect of Zinc on Diarrhoeal Episodes:

Effect of zinc supplementation on duration of diarrhoeal episodes will be assessed using a Cox-survival regression model with a time dependent co-variate. The proportion of episodes lasting more than seven days and hospital admission rates will be evaluated using logistic regression models with duration (7+ days or less than 7 days) and hospital admission (yes/no) as binary dependent variables. The mean number of watery stools will be evaluated by Poisson regression model. The co-variables of interest and interaction terms will be included in the regression models.

Effect on Incidence and Prevalence of Diarrhoea and ARI:

The effect on diarrhoea and ARI morbidity in the one year follow-up period will be evaluated by comparing prevalence rates of diarrhoea and ARI in the treatment and comparison groups. Appropriate statistical methods will be used to account for within child correlations. As some children will have more episodes of diarrhoea than others (some may not have any episode and consequently may not receive any supplementation), stratified and sub-group analysis may be required.

Effect on Growth:

The bi-monthly weight and length measurements will be used to calculate growth velocities and change in z-score weight-for-height, weight-for-age, and height-for-age. Differences in the mean growth velocities and z-scores between the intervention and comparison groups will be compared using appropriate statistical tests.

Study Schedule:

- | | | |
|-----------------------|---|--|
| June 1998 | : | Recruit and train staff, calculate baseline morbidity rates, select treatment and study villages |
| July 1998 - June 1999 | : | Conduct study, collect and process outcome data |
| July 1999 | : | Complete analysis and report preparation |

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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Biography of the Investigators

Biographical Sketch

Name: Abdullah H Baqui, MBBS, MPH, DrPH

Birth date: 03/31/53

Title: Senior Epidemiologist and Head, Child Health Program
Public Health Sciences Division, ICDDR,B

Education:

Institution and Location	Degree	Year conferred	Field of study
Dhaka Medical College, Bangladesh	MBBS	1976	Medicine
Johns Hopkins University, USA	MPH	1985	International Health
Johns Hopkins University, USA	DrPH	1990	International Health

Research and or Professional Experience:

1977-1978 Medical Intern, Dhaka Medical College, Dhaka, Bangladesh
1978-1981 Medical Officer, Matlab Health Research Station, ICDDR,B, Bangladesh
1981-1987 Physician-in-Charge, Clinical Services, Matlab Health Research Station, ICDDR,B
1987-1990 Senior Medical Officer/Assistant Scientist, Department of Epidemiology, ICDDR,B
1990-1994 Head, Research and Evaluation, Urban Health Extension Project, ICDDR,B
1994-1994 Associate Project Director, Urban Health Extension Project, ICDDR,B
1990-1995 Research Associate, Dept of Int. Health, Johns Hopkins Univ.
1996-97 Assistant Scientist, Dept of Int. Health, Johns Hopkins Univ
1997-present Adjunct Assistant Professor, Dept of Int. Health, Johns Hopkins Univ
1994-present Project Director, RISC Project, ICDDR,B
1994-1997 Project Director, MCH-FP Extension Project (Urban), ICDDR,B
1998-present Senior Epidemiologist & Head, Child Health Program, PHSD, ICDDR,B

SELECTED PUBLICATIONS:

Baqui AH, Black RE, Sack RB, Yunus M, Siddique AK and Chowdhury HR. "Epidemiologic and clinical characteristics of Acute and Persistent Diarrhoea in Rural Bangladeshi Children." Acta Paediat Scand Suppl 381:15-21, 1992

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

A community-based, randomized, controlled trial to assess the effect of zinc supplementation in <5 year old Bangladeshi children during diarrhoea on the clinical course of diarrhoea, subsequent diarrhoea and ARI morbidity, and growth

Budget Details

For the period of 14 months (June'98-July'99)

SALARY: LOCAL

Position	Pay Level	# of Staff	% of effort	Monthly Rate	1st Yr. June-Dec'98	2nd Yr. Jan-July'99	Sub-total	Total US \$
Dr. Abdullah H. Baqui	P-5/6	1	37%	9,633	24,949	24,949	49,898	
Dr. Md. Yunus	NO-D/21	1	5%	2,125	744	744	1,488	
Mr. J. Chakrabarty	NO-C/7	1	5%	1,370	480	480	959	
Dr. Shams El Arifeen *	NO-C/7	1	60%	1,370	0	5,754	5,754	
Medical Officer	NO-A/7	1	50%	833	2,916	2,916	5,831	
Data Analyst/Manager	NO-A/1	1	100%	674	4,718	4,718	9,436	
Senior Field Research Office	GS-6/7	1	100%	600	4,200	4,200	8,400	
Secretary Gr-I	GS-6/7	1	100%	600	4,200	4,200	8,400	
Health Assistant	GS-3/7	2	100%	297	4,158	4,158	8,316	
Data Entry Technician	GS-3/7	2	100%	297	4,158	4,158	8,316	
Community Health Worker	Spl Level	16	100%	85	9,520	9,520	19,040	
								\$125,838

LOCAL TRAVEL

Conveyance cost							1,000	
Per diem (for PI at Matlab @100 days)							952	
								\$1,952

SUPPLIES & MATERIALS

Zinc Syrup							6,000	
Computer upgrade, supplies and software							2,500	
Office supplies							3,000	
SUPPLIES & MATERIALS SUB-TOTAL:								\$11,500

OTHER DIRECT COST

Communications							500	
Printing & Publications of forms							2,000	
Service charge (daily wagers)							2,000	
OTHER DIRECT COST SUB-TOTAL:								\$4,500

INTER-DEPARTMENTAL SERVICES

Transport (land & water)							9,000	
Medical Illustration							500	
Xerox, Mimeograph, Library							500	
Estimation of serum zinc levels 300 specimen							1000	
INTER-DEPARTMENTAL SUB-TOTAL:								\$11,000

TOTAL DIRECT COSTS:								\$154,791
OVERHEAD @25%:								38,698
TOTAL PROJECT COSTS:								193,489

* Covered by Johns Hopkins University for 1998

4.5.98
Sr. Budget Officer

CONSENT FORM

Study Title: A community-based, randomized, controlled trial to assess the effect of zinc supplementation in <5 year old Bangladeshi children during diarrhoea on the clinical course of diarrhoea, subsequent diarrhoea and ARI morbidity, and growth

Principal Investigator: Dr. Abdullah H. Baqui, Head, Child Health Program, Public Health Sciences Division, International Centre for Diarrheal Disease Research, Bangladesh (ICDDR,B).

Study location: Matlab, Bangladesh

Elements of informed consent:

Recent controlled trials have shown that zinc supplementation reduces the duration and severity of treated acute and persistent diarrhoeal episodes, reduces subsequent risk of acute and persistent diarrhoea, dysentery, ARI, and improves growth. However, the total available body store of zinc is small and maintenance of a zinc-sufficient status requires regular intake which is not feasible in a program situation. This randomized, community trial is to test a delivery strategy of zinc which is based on the premise that if all episodes of diarrhoea are treated with zinc, the severity and duration of the treated episodes will be reduced. In addition, a positive zinc balance will be maintained in most children which in turn will reduce morbidity and improve growth.

As a parent of a child you are requested to allow your child to participate in this study. The study is sponsored by the ICDDR,B. It is essential that you understand that: i) taking part in the study is entirely voluntary and ii) you may withdraw from the study at any time without loss of benefits to which you are otherwise entitled.

If your child participates in this study s/he will receive the usual diarrhoea care provided by ICDDR,B. In addition, s/he may receive 20 mg elemental zinc per day for 14 days during each episode of diarrhoea. Earlier ICDDR,B studies have clearly shown the safety of zinc supplementation in children during diarrhoea. A study worker will visit your home once every two months to collect data on diarrhoea and ARI morbidity including severity, duration and treatments for current episodes. Dietary data will be collected from a sample of children to assess the dietary intake of zinc. 3 ml blood will be collected once or twice over the study year from a sample of study children for estimation of serum zinc level. Your child may be selected for these components of the study. If your child's age is between 6-11 months at the beginning of the study, a study assistant will measure the weight, length, and mid-upper arm circumference of your child during enrollment and thereafter every other months.

The identity of your child will remain confidential in any publications resulting from this study. The records may be reviewed by representatives of the ICDDR,B as part of their responsibility to oversee this study. By signing this consent document, you agree to such inspection and disclosure.

Consent: The study described above has been explained to me, and I voluntarily consent to participate in it. I, _____, RID _____, age _____ having full capacity to consent do hereby give consent for my child to participate in this study.

Signature of parent: _____ Date: _____

Name of witness: _____ Signature of witness: _____

EVALUATION OF CHILD HEALTH RESEARCH PROPOSAL**Reviewer's name:****Name of proposal:** A community-based, randomized, controlled trial to assess the effects of zinc supplementation in . . .**Name of proposed investigator:** Abdullah Baqui and Robert Black**Date of review** 4/14/98

The USAID Child Health Research Project requires external reviews of all proposals considered for funding. The review serves to ensure high quality of the research supported by USAID, as well as to provide specific suggestions to investigators to assist in revising research protocols. Reviewers comments will be used by USAID personnel in determination of project funding recommendations, as well as by the investigators themselves, who will receive them anonymously.

The proposal seeks funds with which to evaluate the effect of zinc supplementation in children during acute diarrhea on the clinical course of each episode of diarrhea including severity, duration and hospitalization rates, on subsequent morbidity due to diarrhea and acute respiratory tract infections, and on growth of the children. The population to be studied would be children under five years of age in Bangladesh and comparisons would be made between children given oral rehydration therapy as the control group and those given oral rehydration therapy as well as zinc supplementation.

It would have been helpful if the authors had more clearly articulated the difference between this study design and population and the recently published articles demonstrating a clear response to zinc supplementation in the amelioration or prevention of diarrheal disease and on the incidence of respiratory tract infections. A number of studies cited by the authors document a significant preventive effect of zinc supplementation on diarrheal morbidity, both in prevalence and in severity. Other studies cited by the authors also showed that there was a reduction in pneumonia morbidity associated with zinc supplementation. The effects of zinc on growth are well-documented in the literature, most recently by Ninh et al, 1996.

The presence of a well-established, highly evolved method of studying health in this area of Bangladesh would make the study of these hypotheses very feasible. There appeared to be in place a good method for providing health care, for reporting illnesses and deaths, and for facilitating the distribution of the oral rehydration therapy packets and the zinc supplements. One difference proposed by the proposal is to collect information about feeding information including intake of zinc-rich foods as well as foods containing phytates. This seems an important addition to any study of zinc status and disease, but no methods or explanation of how these dietary intake assessments are to be conducted or what data bases would be used in the analysis of the data are given. It also seems a weakness in the experimental design that no method of assessing zinc

status in the children is to be attempted. It is tempting to speculate that an absence of effect to zinc supplementation might be due to sufficient zinc status prior to giving the zinc supplement. It is also not clear how the issue of how not providing a child in a test village with zinc supplements is to be dealt with. The supplement is provided to the child at the time of treatment for diarrhea. If there is no diarrhea then there is no supplement and thus any effect of zinc on growth or respiratory disease would presumably not be possible. Perhaps this is not essential for the testing of research hypotheses, but this could have been explained more clearly.

There seems considerable merit to the ideas included in the proposal. The question of zinc deficiency in developing countries is being recognized as important and the consequences on morbidity and mortality are finally being examined. This study has the potential to extend the findings of the previous studies, but differences between the present study and those which have previously been conducted should be more clearly articulated. Greater description of the methods to be used for some of the assessments would have greatly strengthened the proposal. I do not support funding for the proposal in its present format.

EVALUATION OF CHILD HEALTH RESEARCH PROPOSAL

Name of proposal: A community-based, randomized, controlled trial to assess the effect of zinc supplementation in < 5 year old Bangladeshi children during diarrhoea on the clinical course of diarrhoea, subsequent diarrhoea and ARI morbidity, and growth.

Name of proposed investigators: Baqui et al.

Date of review: April 1, 1998

Comments to authors

I find the design of the study very confusing. There are too many objectives, outcomes, cohorts and data collection procedures. As a first step, the proposal would benefit from a clear description of what exactly the intervention is supposed to be. The description comes only on page 17, and is incomplete. One needs to go back and forth in the proposal to find out additional information on how the study will be carried out. Some kind of figure summarizing the different outcomes, samples and sample sizes, cohorts used, data collection procedures and length of follow up would be useful.

The study is mixing up the therapeutic and preventive effects of zinc supplementation, i.e. it is expecting to obtain preventive effects from a therapeutic intervention given during diarrhea episodes. It is not clear to me that this makes sense. One of the main problems is that the intensity of the intervention (or the total dose of zinc that a child will have received at the end of the intervention) will depend on the number of episodes of diarrhea that this child will have had over the study period. It is well recognized that the peak incidence of diarrhea in children is between 9 and 18-24 months of age. Thus, children in this age range are likely to receive much larger doses of zinc than older or younger children. This makes the group of 0-5 years old an even more heterogeneous group than it already is. Growth rates, morbidity patterns, dietary deficiencies and requirements are too variable within this age range to add yet one more source of variability associated with the nature of the intervention. If the intervention is to be given during diarrhea episodes, I would recommend to restrict the study to a much narrower age range, say 9-18 months old children.

A major design flaw of the study is that the randomization of the intervention is to be done at the village level and all sample size calculations and analysis plans are at the individual level. If the intervention is to be done at the village level, sample sizes should be calculated for the number of villages and of replicates (individual children) within each village. Analyses also would have to take into account that the individuals are not the unit of randomization, but rather the villages are.

Title of Project: A community-based, randomized, controlled trial to assess the effect of zinc supplementation in <5 year old Bangladeshi children during diarrhoea on the clinical course of diarrhoea, subsequent diarrhoea and ARI morbidity, and growth

Responses to Reviewers Comments

The two reviewers made a number of important observations. Their observations were carefully considered and the protocol has been revised as appropriate. There were a few observations that actually did not call for any modification of the protocol but necessitated further clarification. Following are the responses of the reviewers comments which also includes a summary of the changes made:

Reviewer 1:

The first reviewer made the following 4 points.

1. It would have been helpful if the authors had more clearly articulated the difference between this study design and population and the recently published articles demonstrating a clear response to zinc supplementation in the amelioration or prevention of diarrhoeal diseases and on the incidence of respiratory tract infections.

Response: The main difference between the recent trials and this study is that all the recent trials were efficacy trials conducted in a controlled situation and the proposed study is an effectiveness trial which aims to measure the impact of zinc supplementation in a program situation.

The basis of this study is the recent controlled trials that have established the therapeutic effects of zinc supplementation on the duration and severity of acute and persistent diarrhoeal episodes and the preventive effects of zinc supplementation on the incidence of acute and persistent diarrhoea, dysentery, and ALRI. The basis also includes the biological rationale that there is net loss of zinc during diarrhoea and that this may contribute to population zinc deficiency.

While the benefits of zinc supplementation have been demonstrated, how zinc can be delivered in a program situation is not clear. Unlike vitamin A, the total available body store of zinc is small and maintenance of a zinc-sufficient status requires regular intake. This study is based on the premise that if all episodes of diarrhoea are treated with zinc, the severity and duration of the treated episodes will be reduced. In addition, a positive zinc balance will be maintained in most children which in turn will reduce morbidity and improve growth.

2. No methods or explanation of how dietary assessments are to be conducted.

Response: This was a valid criticism and a section on dietary assessment has now been added. A full dietary assessment of zinc intake of all study children is beyond the scope of the study. Data will be collected from a sample of children of various ages drawn from both treatment and control villages. This would help identify any differences in zinc intake in the treatment and control children and would help with external validity of the study.

The following para has been added to the protocol:

Individual dietary data will be collected for a 10% systematic sample of the study children of both the treatment and control villages at baseline and thereafter once every two months. Trained field interviewers will ask each mother of the sampled children to report all foods eaten by her child in the 24 hours preceding the interview. Using pre-measured utensils and spoons, the interviewers will determine and record the types and amount of all foods that were consumed during the previous day. Nutrient contents including zinc content of the foods consumed will be calculated using food composition tables relevant for Bangladesh (Nutrient contents of Indian food. C. Gopalan, National Institute of Nutrition, Hyderabad, India).

3. It also seems a weakness in the experimental design that no method of assessing zinc status in the children is to be attempted.

Response: Previous studies in Bangladesh documented that children during acute phase of diarrhoea are hypozinaemic (Sarker SA et al., 1985) and that zinc supplementation as 20 mg elemental zinc per day for 2 weeks during a diarrhoeal episode significantly increases the mean serum zinc level of supplemented children (Roy SK et al., 1997). However, we agree with the reviewer that it will be useful to have an assessment of zinc status. Accordingly, the following section has been added in the proposal.

Assessment of zinc status in the study children:

Blood will be collected from a sample of the following five groups of study children for estimation of serum zinc level:

- i) Children with acute diarrhoea and from the treatment villages;
- ii) Same children 3 weeks after recovery;
- iii) Age and gender matched children with acute diarrhoea and from the control villages;
- iv) Same children 3 weeks after recovery; and
- v) Age and gender matched healthy control children.

An earlier study in ICDDR,B (Sarker et al., 1985) found mean ($\pm$ sd) serum zinc level of $11.0 (\pm 2.0) \mu\text{mol/l}$ in healthy children and $8.3 (\pm 2.2) \mu\text{mol/l}$ in diarrhoeal children during acute phase. To detect this difference 11 children in each group are needed. The study proposes to measure the serum zinc level of 50 children of each type or a total of 250 children. First, 50 children with acute diarrhoea from the treatment villages (index children)

will be randomly selected over the study year. An age and gender matched child with acute diarrhoea from a control village and an age and gender matched healthy child for each index child will be selected within one week of selection of the index child. About 3 ml blood will be collected from each selected children. All blood samples will be collected in plastic containers carefully washed to make them zinc free. Serum zinc will be measured using an atomic absorption spectrophotometer.

4. It is not clear how the issue of not providing a child in a test village with zinc supplements is to be dealt with...

Response: It is true that the intensity of the intervention will depend on the number of diarrhoeal episodes. The second reviewer also made this point. However, the study is based on the premise that zinc status of many children are marginal who become deficient during diarrhoeal episodes and that if all episodes of diarrhoea are treated with zinc, the severity and duration of the treated episodes will be reduced. In addition, a positive zinc balance will be maintained in most children which in turn will reduce morbidity and improve growth.

It is true that if there is no diarrhoea then there is no supplement. However, there are very few children of this age range in this population who have no diarrhoea. In addition, if they have low rates of diarrhoea by our hypothesis they may need less supplemental zinc. Conversely, if they have many episodes, they need to receive more zinc. Finally, what we are looking for is an efficient delivery strategy that has both therapeutic and preventive benefits. This is an effectiveness trial, so the impact at the population level is what we are interested in. However, we plan to do sub-analysis restricting the analysis to children with diarrhoea and this was mentioned in the protocol.

Reviewer 2:

The second reviewer made the following 4 point:

1. Too many objectives, outcomes, cohorts and data collection procedures..... Some kind of figure summarizing the different outcomes, samples, sample sizes, cohorts used and data collection procedures would be useful.

Response: Some of the less important outcomes have been deleted. Table 1 summarizes the different outcomes, samples, sample sizes, cohorts used and data collection procedures.

2. One of the main problems is that the intensity of intervention will depend on the number of episodes of diarrhoea a child will have over the study period.

Response: This point has already been addressed.

3. It is well recognized that the peak incidence of diarrhoea in children is between 9 and 18-24 months of age. Thus, children in this age range are likely to receive much larger doses of

zinc than older or younger children. This makes the 0-5 years old an even more heterogeneous group than it already is. i would recommend to restrict the study to a much narrower age range, say 9-18 months old children.

Response: The study will be carried out at Matlab, Bangladesh. It is true that in this population, diarrhoea rate peaks in 6-11 month old infants (6.2 episodes/child/year). However, diarrhoea rates are also high in young infants below 6 months of age (5.1 episode/child/year) as well as in older children. The rates in the 3rd and 4th year of life are 4.3 episodes/child /year and 3.6 episodes/child/year respectively. In addition, an earlier study in Bangladesh in children 6 months to 6 years observed low serum zinc level in children during acute phase of diarrhoea. Serum zinc concentration in children in this study was not related to age. Thus, this study in children less than 5 years of age is justified.

4. A major design flaw of the study is that the randomization of the intervention is to be done at the village level and all sample size calculations and analysis plans are at the individual level.

Response: This criticism is not valid. When interventions are allocated to communities (in this case villages) but individuals within the communities are subject of interventions, sample size determination can be done in two ways. One option is to consider communities as unit of observation. However, for calculating the required number of communities, the community level incidence rates (or proportions or means) and variance estimates are required and such data are often not available. Alternatively, sample size can be calculated for individuals but then the sample size needs to be multiplied by the appropriate 'design effect'. The design effect will be 1 if there is no heterogeneity between communities in the outcomes of interest, in the sense that the variation between the community-specific rates or means is no more than would be expected to occur by chance due to sampling variations. For most outcomes, however, there will be real differences between communities (i.e., the design effect will be >1), and in these circumstances the required study size will be greater than with individual allocation. We calculated sample size for individuals but assumed a design effect of 3 for most outcomes. Furthermore, we plan to allocate communities into intervention and control using a matched (paired) design. With a paired design, at least six communities are required in each group (Methods for field trials of interventions against tropical diseases, eds. Smith PG and Morrow H, WHO, 1993). We plan to have as many as 20 villages in each group. Thus, the sample size should be adequate.