CONTROLLED TRIAL TO PREVENT ACUTE LOWER RESPIRATORY TRACT INFECTION AND DIARRHOEA WITH ZINC SUPPLEMENTATION IN CHILDREN BELOW 2 YEARS OF AGE

W. ABDULLAH BROOKS
AND OTHERS

Attachment 1.
(FACE SHEET)

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Date 03.06.98

Principal Investigator W. A. Brooks

Application No. 98-011

Title of Study Controlled Trial to Prevent ALRI/Diarrhoea with Zinc Supplementation < 2y/o

Trainee Investigator (if any) _____

Supporting Agency (if Non-ICDDR,B) _____

Project status:
(X) New Study
( ) Continuation with change
( ) No change (do not fill out rest of form)

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

1. Source of Population:
   (a) Ill subjects — Yes No
   (b) Non-ill subjects — Yes No
   (c) Minors or persons under guardianship — Yes No
2. Does the study involve:
   (a) Physical risks to the subjects — Yes No
   (b) Social Risks — Yes No
   (c) Psychological risks to subjects — Yes No
   (d) Discomfort to subjects — Yes No
   (e) Invasion of privacy — Yes No
   (f) Disclosure of information damaging to subject or others — Yes No
3. Does the study involve:
   (a) Use of records, (hospital, medical, death, birth or other) — Yes No
   (b) Use of fetal tissue or abortus — Yes No
   (c) Use of organs or body fluids — Yes No
4. Are subjects clearly informed about:
   (a) Nature and purpose of study — Yes No
   (b) Procedures to be followed if used alternative risks — Yes No
   (c) Physical risks — Yes No
   (d) Sensitive questions — Yes No
   (e) Benefits to be derived — Yes No
   (f) Right to refuse to participate, withdraw from study — Yes No
   (g) Confidential handling of data — Yes No
   (h) Compensation for treatment where risks are involved in particular procedure — Yes No
5. Will signed consent form be required:
   (a) From subjects — Yes No
   (b) From parent or guardian (if subjects are minors) — Yes No
6. Will precautions be taken to protect anonymity of subjects — Yes No
7. Check documents being submitted herewith to Committee:
   - ___ Umbrella proposal - Initially submit an overview (all other requirements will be submitted with individual studies).
   - ✓ Protocol (Required)
   - ✓ Abstract Summary (Required)
   - ___ Statement given or read to subjects on nature of study, risks, types of questions to be asked, and right to refuse to participate or withdraw (Required)
   - ___ Informed consent form for subjects
   - ___ Informed consent form for parent or guardian
   - ✓ Procedure for maintaining confidentiality
   - ___ Questionnaire or interview schedule *

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

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

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

W. A. Brooks
Principal Investigator

Trainee

International Centre for Diarrhoeal Disease Research, Bangladesh **FOR OFFICE USE ONLY**

Protocol No:

Date:

RESEARCH PROTOCOL RRCApproval: Yes/ No Date:

ERC Approval: Yes/No Date:

1. Title of Project (Do not exceed 60 characters including spaces and punctuations)

Controlled Trial to Prevent Acute Lower Respiratory Tract Infection and Diarrhoea with Zinc Supplementation in Children Below 2 Years of Age

2a. Name of the Principal Investigator(s) : 1) W. Abdullah Brooks, MD, MPH; 2) : ASG Faruque, MB,BS, MPH; 3) MA, Wahed, Bsc; 4) George Fuchs, MD; 5) Robert Black, MD, MPH; 6) Mathuram Santosham, MD, MPH; 7) Abdullah H. Baqui, MBBS, Ph.D. **2b.** 1) Health & C)hild Survival Advisor; 2) Scientist; 3) Medical Biochemist 4) Director of Clinical Sciences Division. 5) Prof. & Chair Int'l Health, JHU. 6) Prof. Int'l Health, JHU; 7) Sceintist **2c. Qualifications**

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

• **Contact Address of the Principal Investigator**

4a. Office Location:

GPO 120 Mohakhali

Dhaka, 1000

4b. Fax No: 883.116

4c. E-mail: abrooks@citechco.net

4d. Phone / Ext: 871.751 X 2327

5. **Use of Human Subjects** **Yes** [X] **Yes** **No** [ ]

5a. Use of Live Animal [ ] **No** [X]

5b. If Yes, Specify Animal Species

6. **Dates of Proposed Period of Support**

Two years, to begin as soon as funding is available.

7. Cost Required for the Budget Period

7a. Ist Year ($):169,214 2nd Year ($):97,497 3rd Year: *N/A*

7b. Direct Cost ($)**233,957 **Total Cost ($)266,711 of which $50,000 is being sought from SDC. Co-funding sought for remainder.

8. 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.

George Fuchs, MD

Name of the Division Director | Signature | 4 June 98 Date of Approval

1. 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.

10. Signature of PI

W. Abdullah Brooks

Date: 04 Jun 98

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[X] Check here if appendix is included

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

Principal Investigator **W. Abdullah Brooks, MD, MPH**

Project Name **Controlled Trial to Prevent Acute Lower Respiratory Tract Infection and Diarrhoea with Zinc Supplementation in Children Below 2 Years of Age**

Total Budget $266,711 Beginning Date As soon as funding is available, for two years. Ending Date

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ABSTRACT

The World Health Organization has identified diarrhoea and acute lower respiratory tract infection (ALRI) as high priority areas for intervention in order to reduce childhood morbidity and mortality. Prevention would address both of these objectives, and would have high cost benefit. Presently, there is no general prophylactic agent with demonstrated efficacy against these two diseases in this age group. The most vulnerable age group to ALRI and diarrhoea is that below two years of age. Limited data from community-based studies have reported preventive benefit from daily doses of zinc in children against both diarrhoeal and, more recently, acute lower respiratory tract diseases. Previous studies have not concentrated on the under two-year age group, nor has this group been shown to derive a clear benefit from zinc. We hypothesise that a relative zinc deficiency exists in the vast majority of infants below 2 years of age in developing countries and results in impaired cellular immunity needed to resist ALRI and diarrhoeal infections. The effect may be dose-dependent. We propose to supplement infants below two years of age with two different dosing regimens to determine whether or not zinc supplementation in young infants can lessen the incidence of ALRI and diarrhoeal disease, and to investigate the possibility that such benefit is dose-related. In so doing, we will get an estimate of the community incidence of ALRI in the under-two year old population, where disease-specific morbidity and mortality is highest. The results of this study will have major implications for the control of diarrhoeal disease and ALRI around the world.

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

Name	Professional Discipline/ Specialty
Role in the Project	
1. W. Abdullah Brooks, MD, MPH	Preventive Medicine, Paediatrics
2. ASG Faruque, MB,BS; MPH	Scientist, Epidemiologist
3. MA Wahed	Laboratory Scientist
4. George Fuchs, MD	Gastroenterologist, Paediatrics
5. Robert E. Black, MD, MPH	Professor & Chair, Int'l Health, JHU
6. Mathuram Santosham, MD, MPH	Professor, Int'l Health, JHU
7. Abdullah H. Baqui, MBBS, Ph.D	Scientist, Epidemiologist

DESCRIPTION OF THE RESEARCH PROJECT

Hypothesis to be tested:

1. Zinc deficiency is highly prevalent in young Bangladeshi infants and reduces resistance to ALRI and diarrhoeal infections.
2. Zinc supplements will lower the incidence of ALRI and diarrhoeal diseases.
3. A once-weekly total dose is as efficacious as the same dose divided daily in reducing the incidence of ALRI and diarrhoea.

Specific Aims:

Objective:

1. To determine if zinc supplementation in zinc deficient young infants reduces the incidence of ALRI or diarrhoeal disease.
2. To investigate if the preventive effect is affected by dosing schedule.

Specific Aims

1. To determine if 10 mg of elemental zinc QD X 6-mo vs placebo decreases ALRI/diarrhoeal disease incidence.
2. To determine if 70 mg of elemental zinc QWk X 6 mo vs placebo decreases ALRI/diarrhoeal disease incidence.
3. To compare the efficacy of 10 mg once daily to 70 mg once weekly elemental zinc in preventing ALRI and diarrhoea.
4. To examine the effect of age on diarrhoea prevention.

Secondary Aims

1. To estimate the effect of 6 mo Zn supplementation on growth.

2. To measure the effect of 6 mo Zn supplementation on Cu status.
3. To measure the effect of 6 mo Zn supplementation on Zn status.

Rationale:

SEE DESIGN, RATIONALE IS ITEMISED BY SPECIFIC AIM.

Background of the Project including Preliminary Observations

BACKGROUND

Acute lower respiratory infection (ALRI) is a leading cause of morbidity and mortality in children below five years of age(1). Pneumonia accounts for most of these deaths, and thus defines ALRI. In fact, 25-33% of the deaths among children under 5 years are attributable to pneumonia (4 million deaths/year world-wide); two thirds of which occur during infancy and over 90% of which take place in developing countries(1,2). Acute respiratory illness (ARI), overall, ranks as one of the two leading causes of hospitalisation of children in Bangladesh(3). **It has been reported that the incidence of ALRI is 8-9 episodes/child/yr during the first 2 years of life, dropping to 3-4 episodes/yr in 2-4 year olds(7). Thus, the susceptibility to ALRI in children under two years is more than twice that of older children.** While overall child mortality in some developing countries has declined among younger age groups, the number of deaths attributable to ALRI has remained unchanged, suggesting an increasing importance of ALRI as a cause of death among young children(8). A pooled estimate from a recent meta-analysis concluded that a case management approach to children with rapid breathing could reduce total infant mortality by 20% and mortality for all children under five years by 25%(1). It further argued that a case management approach that relies principally on early diagnosis and appropriate antibiotic therapy could reduce pneumonia case fatality by as much as 50%(1). The most recent figures for ALRI incidence in Bangladesh are from a rural setting(79). Incidence for children below age 24 months ranged 0.2 – 0.5 episodes/child/year, averaging 0.3 episodes/child/year.

Diarrhoeal disease is also a worldwide problem of major importance in developing countries. **One fourth of avoidable deaths in hospitalised children is a result of diarrhoea, and 80% of these deaths occur in children <2 years of age (9).** The numbers of children dying each year from diarrhoea has decreased over the last decade from five to three million, in large part due to the widespread use of oral rehydration solution (ORS) (9). Despite the overall reduction in mortality, the persistently high mortality rate emphasises the need to develop additional strategies. The use of ORS to correct fluid and electrolyte abnormalities and antimicrobials to treat selected diarrhoeal diseases, including cholera and Shigella, remain central to the management of childhood diarrhoea in developing countries.

Despite advances in diagnosis and treatment, the total number of deaths attributable to both ALRI and diarrhoea in children below five years of age is over 6 million, or 38% of all deaths(29). Recent data from Bangladesh indicate that ALRI is the most important cause of death in children under age 5 years, accounting for about 18% of all deaths(75). The effect is greater for younger age groups, where 30% of deaths in the one to 11 month age group is attributed to ALRI, while it accounts for 11% of deaths in the neonatal period, second only to

neonatal tetanus. In the post-neonatal young infant age group, an additional 8.6% of young infant deaths were attributable to ALRI in combination with some form of diarrhoeal disease, while a further 10.6% of deaths were possibly due to ALRI, with or without concomitant infections. Thus, in Bangladesh as much as 50% of mortality among young infants is possibly attributable to ALRI(75). Furthermore, the under-five mortality trend has increased slightly from 18% in the period 1989-91 to 21% in the 1992-94. Understandably, WHO identifies ALRI and diarrhoea among the five priority areas in childhood illness for intervention as a means to decrease overall child morbidity and mortality(29).

Treatments have been devised for both ALRI (antibiotics, supportive care) and diarrhoea (oral rehydration therapy) (30,31,32). Their specific purpose has been to reduce mortality. Still, case fatality for both diseases remains high. Even if mortality can be reduced, these treatments will have little impact on morbidity. Prevention alone reduces the incidence of morbidity, and may reduce mortality. Therefore, prevention must be the cornerstone of further efforts against these two priority diseases.

Vaccine-based prevention has had an impact on many but not all causes of acute pneumonia. At present, only a small proportion of all pneumonia is effectively prevented (35,36). Clearly, vaccine utilisation in the global community could be vastly improved, but cost and logistics are obstacles(34). Pneumococcal, RSV and rotaviral vaccines promise to make further reductions in the incidence of ALRI and diarrhoea. However, these interventions will face the same obstacles of cost and maintenance of cold chain integrity that have hampered existing vaccination programmes. Assuming that these vaccines are 100% efficacious and effective, a rotaviral vaccine could reduce diarrhoeal morbidity by no more than 46%(19). However, the currently licensed vaccine is only 80% efficacious. Similarly, an optimal RSV vaccine could reduce ALRI morbidity by no more than 40%(37). The efficacy of present pneumococcal vaccines in Asia, including the Subcontinent, is uncertain at this time(38,39) and trials are underway to address this issue. Clearly, there is a role for additional safe, inexpensive, easily administered preventive measures that can be integrated into existing childhood illness management strategies, and that can be applied to the most vulnerable age groups, irrespective of disease aetiology.

Cellular Immunity and Malnutrition

The link between nutrition and immunity is now recognised. One study stated that 56% of all deaths among children 6 to 59 months old had malnutrition as a potentiating factor, 83% of which was attributable to the mild-moderate form(74). Recent studies have reported a link between even sub-clinical malnutrition and impaired cellular immunity, and indicate that impaired cellular immunity is a risk factor for development of ALRI(7) and diarrhoeal disease(40,41). Several studies have demonstrated an association between malnutrition and the incidence and severity of pneumonia(7,42,43). Malnutrition has also been shown to increase the risk of pneumonia-related mortality(1,44). One study reported that the risk of ALRI-related death for first, second and third degree malnutrition was 4.4, 11.3 and 27.0, respectively, compared to children without malnutrition(45). Other studies have reported similar increases in mortality risk for malnourished children(46,47).

Host factors are also major determinants of diarrhoea-associated morbidity and mortality(20). Indeed, the interaction between diarrhoeal disease and malnutrition, which coexist in children from the developing world, extracts the greatest cost in productivity and life(21,22). Apart from inadequate energy intake, deficiencies of several specific nutrients,

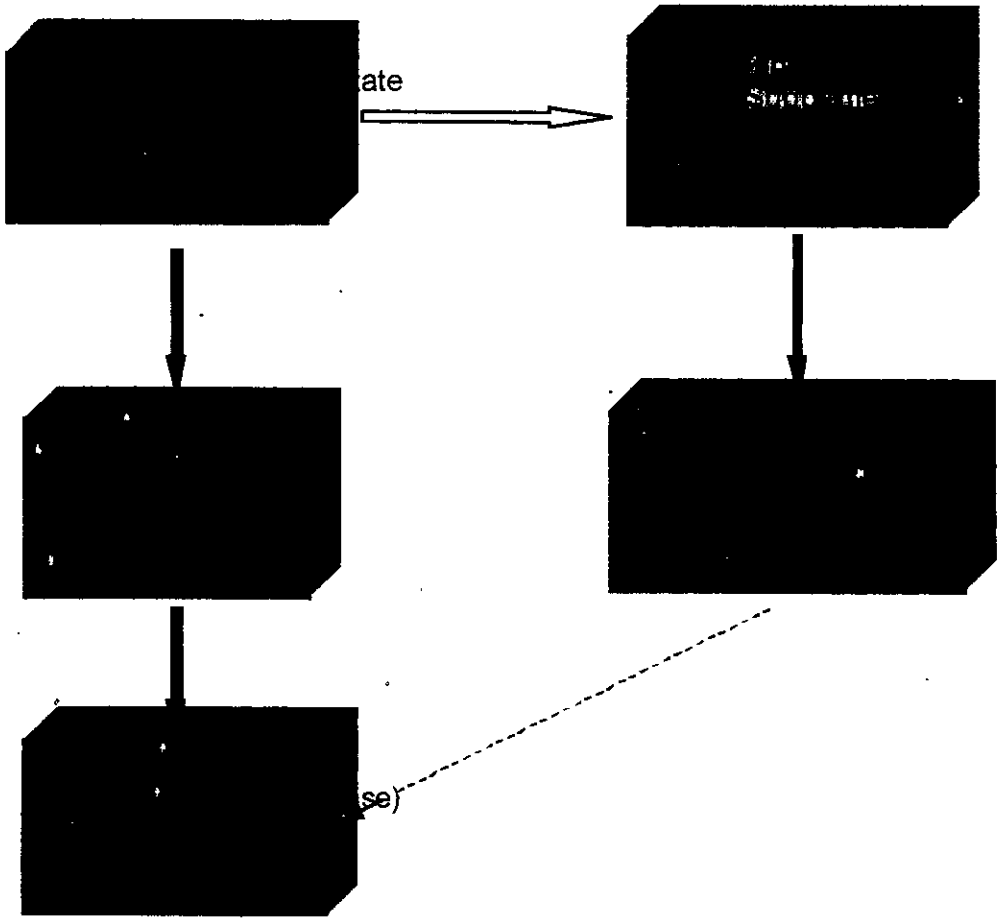
including zinc, have been implicated. Importantly, zinc deficiency is exacerbated by diarrhoea, which is often associated with gross increases in faecal zinc loss (23).

Zinc, Immunity and Morbidity

Current evidence on the interaction between nutritional status, immunity and disease has underscored the importance of cellular immunity (7), which has been documented in malnourished children(7,43,48). Evidence suggests that the impaired cellular immunity in malnourished children may be due, in part, to zinc deficiency since the latter is associated with impaired cellular immunity (7,42) and correction of zinc deficiency is associated with a reduction in the incidence and prevalence of diarrhoeal disease (24,25,26,27,48,49), and a significant reduction in the incidence of respiratory infection(42,43). Indeed, there is evidence of a correlation between the severity of malnutrition and the magnitude of the effect on persistent diarrhoea from zinc supplementation(50).

Figure 1.

Overview of ALRI/Diarrhoea Prevention Trial Hypothesis



Zinc Deficiency Linked to Increased Incidence ARI and Diarrhoea

Zinc deficiency has been reported among young children in many developing countries(26,43,49) with prevalence as high as 38 - 50%(27,28). Both diarrhoea and ALRI have been associated with exacerbated hypozincaemia, some of which may be attributable to accelerated zinc loss in both diseases(23,51,52). Therefore, these children may suffer a double insult. Not only do their inadequate zinc stores render them more susceptible to two highly prevalent infectious diseases, but their depressed zinc status is possibly made worse by the very infections for which zinc is needed to mount a cellular immune response and to resist future infection. This sequence could create a loop or cycle between zinc deficiency, impaired cellular immunity and infection, with significant deleterious effects on the growth and survival of young infants.

Efficacy of Zinc as a Preventive Agent in Zinc Deficient Children

It is clear from the above that host factors are major determinants of ALRI and diarrhoea-associated morbidity and mortality, and that nutritional status is one of the most critical host factors, affecting both immunity and resistance to infection.

Community-based controlled trials that have investigated the preventive effect of zinc have reported reductions in the incidence of both ALRI and diarrhoeal disease in children(24,25,26,27, 28,42,43,48).

ALRI A recent study reported a 45% (95% CI = 10-67%) reduction in the incidence of pneumonia among children aged 6 to 35 months old given zinc supplements over a six month period(42). Another recent study reported reductions in pneumonia incidence among growth-retarded children aged 4 - 36 months given zinc supplements over 5 a month period (43). Two studies found no effect on ALRI incidence(26,49).

Diarrhoea One study reported a 26% lower incidence and a 35% lower prevalence of diarrhoea for children > 11 months old in a six month zinc supplementation trial(27). Two other recent studies reported diarrhoeal incidence reduction rates between 36% and 71%(43, 49).

Only one of these trials included children below six months of age(43), but the sample size was too small to draw any conclusions on this sub-group. All studies included children in older age groups, for whom ALRI and diarrhoeal disease incidence and mortality is substantially lower. Another of these studies only observed significant reduction in diarrhoea incidence among children one year of age and above(27, Black personal communication). Based on available epidemiology, reductions of incidence among the under two-year age group, the most vulnerable population, would have the biggest effect on ALRI and diarrhoeal disease. Mortality data on children presenting to our Centre indicate that 57% of deaths occur in children below one year of age (APPENDIX 1).

Mortality Data for Oct96-Sep97

Figure 2.

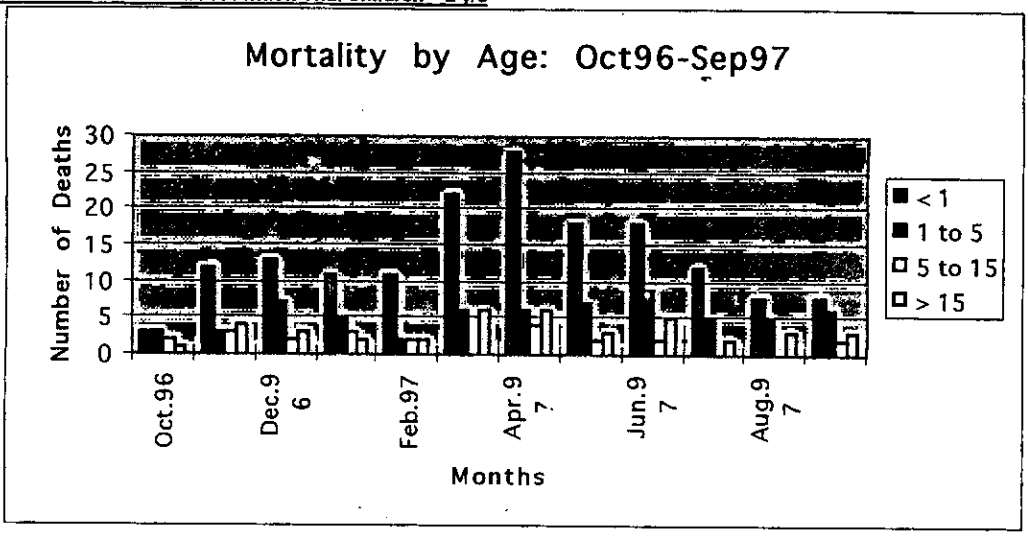
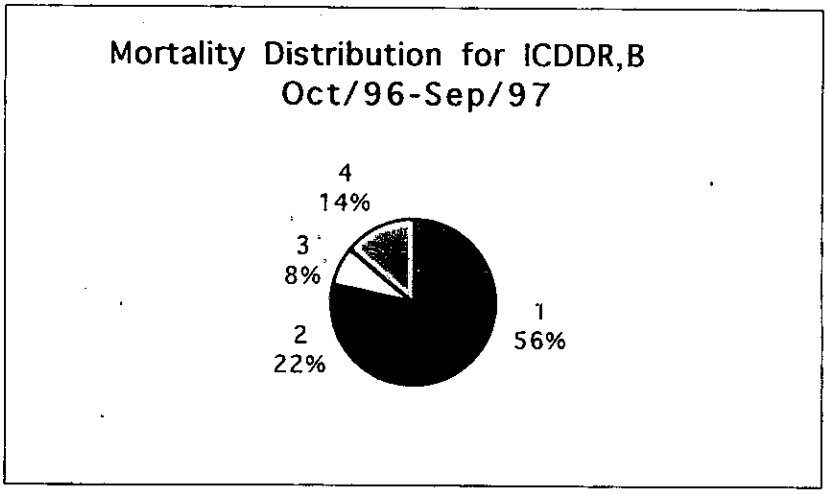


Figure 3.



*LEGEND
Group 1 = < 1 year old
Group 2 = 1 - 5 year old
Group 3 = 5 - 15 year old
Group 4 = > 15 year old

Mortality among children under two years comprises the majority of the remaining deaths of the under five-year age group (data not shown). **These findings are consistent with other data on this age group and indicate that the children under age two years are at the greatest risk for adverse outcome and most in need of an effective intervention(7,9).** Therefore, data on the interaction between zinc and disease incidence specifically in young

infants are inadequate. Most studies of zinc supplementation, to date, were not designed to test the effect of zinc in the under two-year age group. Moreover, attempts have not made to ascertain if their lack of response to zinc, observed in those studies in which young infants have been included, is dose-related. Finally, since there are data to suggest that certain children experience preventive benefits from zinc, studies that explore the feasibility and efficacy of practicable dosing strategies are needed. This study will provide the necessary data to allow national and international ALRI and diarrhoeal disease control programmes to set policy and devise programmatic strategies concerning the routine use of zinc in infants below 2 years of age who are zinc deficient.

Zinc Dosing

The amount of zinc to be used is a question of fundamental importance. Most studies on ALRI used 10 mg/day of elemental zinc, while many diarrhoeal disease studies, particularly on treatment effects, have used approximately 20 mg or more of elemental zinc per day. This latter dose is twice the US recommended daily allowance (RDA) for children one to eleven years of age, and four times the RDA for children under one year(55). While an inadequate dose might result in treatment failures, there is physiological evidence that excessive amounts of zinc can also be harmful(56,57). For example, depressed fungicidal activity consistent with impaired monocyte function, and increased incidence and duration of impetigo have been observed in infants receiving 1.9 mg per kg per day of elemental zinc in consumed formula, compared to control infants receiving 0.35 mg per kg per day(58). It is unlikely that the dosing regimens under study are toxic, and indeed clinical toxicity has not been documented in young infants in any of the ALRI or diarrhoea supplementation studies to date. At present, very little is known about the zinc needs of very young infants in countries with a high prevalence of zinc deficiency. A question of practical importance related to dosing is frequency. Less frequent dosing that results in comparable effects would have logistical benefits, cost savings and might enhance compliance. This alone warrants an investigation of the efficacy of less frequent dosing.

Zinc Interaction with Other Micronutrients

Both zinc and copper are absorbed in the small intestine(59). Zinc inhibits copper absorption, but not vice versa except perhaps at very high copper doses. The need for copper might regulate absorption, with intestinal cell metallothionein (MT) involved in the regulation. Dietary zinc, but not copper, induces synthesis of intestinal MT, even though copper has a higher affinity for MT than does zinc. With increased zinc intake and a resultant increased MT, copper displaces zinc from MT, becomes chelated to enterocyte MT and is excreted in the faeces following desquamation of the enterocyte. This negative effect of zinc on copper balance is exploited in the treatment of Wilson's Disease, an hereditary disorder of copper metabolism manifested by excessive organ copper accumulation (60). Festa *et al* reported reduced copper retention in a group of healthy volunteers who were fed 18.5 mg of elemental zinc per day (3.5 mg above the RDA) for two weeks(61). This study demonstrates that an intake of zinc only slightly above RDA, even short-term, is capable of interfering with copper metabolism. Therefore, zinc supplementation may aggravate marginal copper deficiency(62).

Copper deficiency has been shown to coexist in childhood malnutrition(63,64,65). The usual features of copper deficiency; anaemia, leukopaenia, and neutropaenia, may also include osteoporosis and pathological fractures resembling those of scurvy; presumably because copper is essential for the formation of cross-linkages in collagen and elastin(66,67). These

studies also reported copper to be a limiting growth factor in Chilean children recovering from malnutrition and essential to maintain normal immune function. Thus, the extent to which a prophylactic zinc regimen might contribute to physiological or clinical copper deficiency is a question of direct and immediate relevance to disease prevention.

At two recent zinc symposia, one at Johns Hopkins University in Baltimore, Maryland during November, 1996 and another at the International Centre for Diarrhoeal Disease Research, Bangladesh in Dhaka on 15-16 May, 1997, an international expert panel agreed that further research on the efficacy of zinc in the treatment of AWD in children is needed before universal recommendations can be made(73). With regard to the interaction between zinc and copper, it was agreed that assessment of copper status should be included in new zinc supplementation trials. Thus, there is a consensus of expert opinion that further research is needed on: 1) zinc dosing and; 2) interaction with other micronutrients, specifically copper.

Risks and Benefits of This Project

The potential benefits from this trial of oral zinc supplementation against *two* WHO-priority infectious diseases in young infants are practically unrivalled. These benefits extend to both the child (and its family) and the Centre (the health provider community). To the child and family, the intervention: may be efficacious as a prophylactic against a broad spectrum of infectious agents, possibly effective in the most vulnerable period of the infant's life, and may reduce the proportion of reactive airways disease that is triggered by respiratory infection. Furthermore, there may be an interaction with other primary prevention measures; cellular immunity may improve, for example, enhancing the child's immunological response to vaccines. The benefits may extend beyond immunity to other parameters of the child's wellbeing, such as improved nutritional status and growth - effects that other disease prevention measures like vaccines cannot offer. The intervention will not interfere with present therapeutic regimens for either ALRI or diarrhoea. The risks from this trial include: possible mild gastrointestinal irritation from the zinc gluconate(65); depression of copper status, which to date has not been reported, and; the risk of infection from non-sterile blood drawing technique.

The potential benefits to the health provider community include that it:

1. Addresses *two* WHO-priority diseases (ALRI, Diarrhoea) simultaneously;
2. Addresses *two* expert panel-identified issues in zinc:
3. Dosing;
4. Effect on copper status;
5. Seeks to intervene in the most vulnerable age group, < 2 year olds, hence could result in high cost benefit as a preventive measure;
6. Is safe (insofar as available data suggest);
7. Would have benefits other than immunological, i.e., nutritional and growth effects;
8. Intersects with new and emerging areas of interest such as IMCI, and will deepen our expertise in the area of ALRI, which is a major IMCI component;
9. Will provide data on Bangladeshi infants that no one presently has and which are relevant, if not vital, to our new and emerging areas of interest. These data include ALRI incidence in urban infants, and (new data on) diarrhoea incidence;
10. Provides capacity building for national staff in an emerging and important area, ALRI, which will continue to have benefits to the Centre after the project is completed.

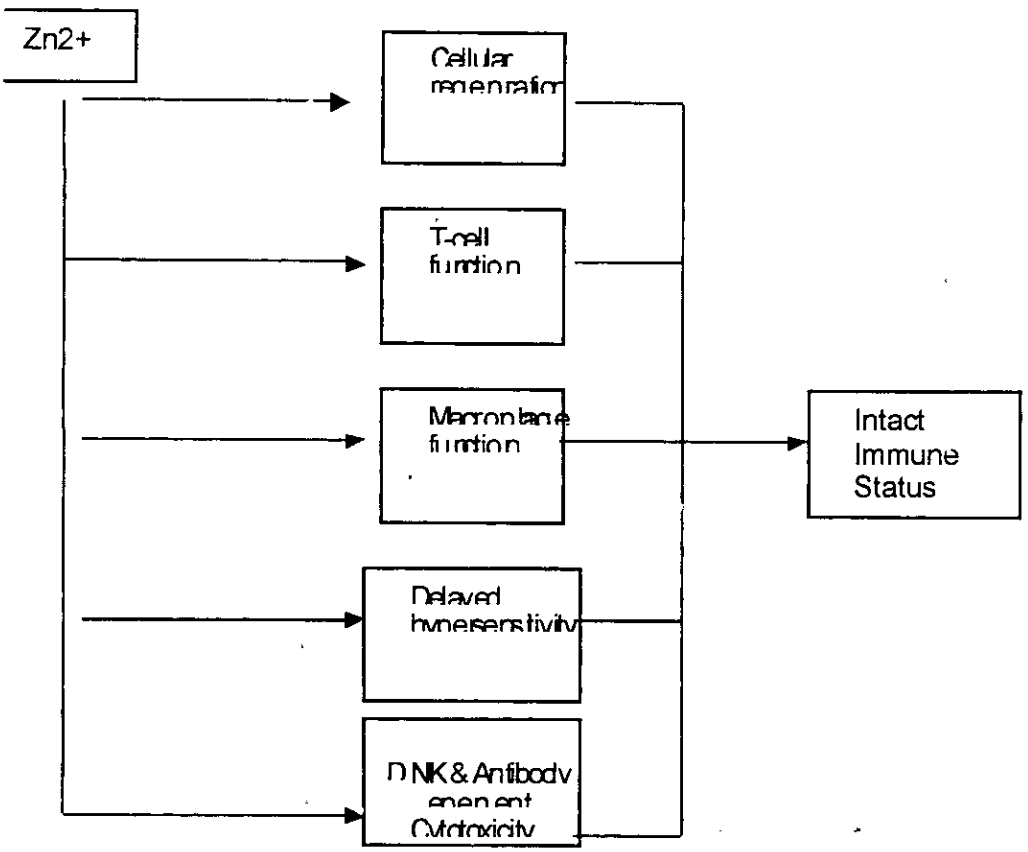
General Goal and Significance

ALRI is the leading cause of infection in infants and, taken together with diarrhoeal disease, accounts for about 40% of all infant mortality. Our long-term goal is to reduce the incidence of community-acquired ALRI and diarrhoea with a safe preventive agent that will not impair the effectiveness of other prevention strategies.

Theoretical Model (Figure 4.)

Zinc deficiency: (i) impairs cell regeneration (due to its role in metalloenzymes, and other metabolic pathways); (ii) impairs immunocompetence with reduced cell mediated immune response, decreased T- lymphocytes, and abnormal T-Helper and/or suppresser cell functions(11), (iii) impairs macrophage function(11), thus depressing the delayed hypersensitivity reaction; (vi) reduces the natural killer cells and antibody dependent cytotoxicity(12), (v) impairs epithelial barrier functions. We believe that the combined effect increases the risk of infection in an infant exposed to ALRI and diarrhoeal pathogens.

Figure 4.



Research Design and Methods

Design: A community-based double-blind placebo controlled preventive trial.

Specific Aims 1 and 2. *Rationale.* The under-two year old population represents the most vulnerable to ALRI and diarrhoea, has been shown to be zinc deficient in the developing

world, including Bangladesh, and zinc may boost immune function and thereby reduce the incidence of these two diseases. *Protocol.* Infants will be recruited from two adjacent communities (see Population, below) with a combined total population of 44,000. A request to participate will be made at the first home visit during the mapping phase. An intake interview and baseline physical examination will be made during the first interview on all willing participants. Infants will then be randomised to one of three groups (TABLE 1). Group A will receive 10 mg of elemental zinc once daily, Group B 70 mg of elemental zinc once weekly followed by six days of placebo supplement and Group C will receive placebo.

TABLE I

1. Age	2. A	3. B	4. C
5.	6. Zinc 10 mg/day	7. Zinc 70 mg/week	8. Placebo
9. 2 – 11 mo	10.282	11.282	12.282
13. 12-23 mo	14.282	15.282	16.282
17. Total	18.564	19.564	20.564

Community Follow-up Enrolled infants will be followed in the community twice weekly. The bi-weekly visits will have two objectives: 1) to check their compliance with taking their supplements; 2) to gather data on intercurrent ALRI or diarrhoeal episodes. Parents will be asked about signs of ALRI or diarrhoeal illness for each of the days since the previous visit. Each infant's respiratory rate will be counted twice for one full minute and the mean recorded by the health worker on each visit. The medical officer will visit any infant noted by the health worker to have signs of illness, as defined in Methods,, and a clinical diagnosis will be made. Infants with non-severe illness (see Definitions in Methods) will be managed as outpatients according to the protocol (Methods). Infants with severe illness will be referred to ICCDR,B hospital, Dhaka for treatment. **Thus, diagnosis of illness, and therefore determination of incidence, will be clinical.**

Compliance check Parents will be given an amount of supplement in excess of the weekly allotment. The residual amount will be measured on the twice-weekly visits and on random spot checks by the health worker and compared with the amount that should remain if the patient were in compliance. Those subjects not in compliance will be noted, and their parents will be encouraged to keep to the study agreement.

Data Demographic data will be *categorical.* Univariate analysis using two by two tables and Chi Square tests will be performed between outcome (ALRI and diarrhoeal illness) and each covariate to assess interaction. Multivariate analysis will be done to control for confounding. A final model of high risk will be determined by logistic regression. Morbidity data will be *continuos. ALRI-associated*; episodes of age-specific tachypnoea, chest indrawing, fever. *Diarrhoea-associated*; episodes of three or more loose watery stools per day or bloody mucoid stools. These will be calculated as **incidence** (mean frequency of occurrence during the study period per group), and **prevalence** (mean duration of symptoms by treatment

group). Analysis type: Kaplan-Meier survival curve. Treatment and placebo group data will be compared by logrank test, to test the null hypothesis that the groups are the same with respect to frequency and duration of illness (and hospital stay). Hazard ratio will be performed to compare the relative frequencies and duration of illness times between groups. Modelling will be performed by Cox Regression.

Difficulties and Alternatives 1) Reporting bias. Surveillance of the enrolled infants will rely on parents' report and recall. The greater the interval between visits, the greater the risk of losing information. Mild illness could be missed by the parent and be sub-clinical at the time of the health worker visit. This could have two consequences. First, it could cause an underestimation of true disease incidence, possibly leading us to over-estimate the effect of zinc on disease rate. Second, it could cause an over-representation of more severe disease, possibly leading to an under-estimation of the effect of zinc on disease severity. We will compensate for reporting bias by physical inspection of all children, irrespective of the infant's reported condition. 2) Missed visits. Infants may not be present at the time of the health worker visit. Any infant not present will be re-visited by the healthworker within 24 hours of that scheduled visit. A tally of missed visits will be kept and included in the analysis. 3) Missing information. Certain data, such as past medical history, immunisation history, may be difficult to ascertain. Information that can be verified by records will be (such as immunisation cards). Further, where there is record-report discordance, the written record will be entered into the data set.

Specific Aim 3. *Rationale.* To assess the relative efficacy of a less intensive, therefore easier to administer, dosing regimen, which would have significant programmatic implications. *Protocol.* Infants will be randomised to one of two treatment groups. Group A will receive 10 mg per day. Group B will receive 70 mg once per week, followed by six days of placebo. Each infant will receive a one month supply of syrup in two measured bottles, 30 ml and 120 ml, respectively. The bottles will be scored to the nearest 1 ml. Mothers will be instructed to give one dose (5 ml) per week (on a specific day) from the small bottle followed by daily doses (5 ml per day) from the large bottle. The bottles will be labelled with the infants' enrolment code. In order to detect treatment group differences, power was set at 90% in sample size calculations, and drove sample size. The magnitude of effect for ALRI and diarrhoea are the conservative estimates published from previous studies and cited in the Background section. *Data.* The outcome will be morbidity data and will be analysed as described above. *Difficulties and Alternatives.* 1) We will only be able to measure the efficacy of single weekly dosing, not its effectiveness, since the weekly dose will be masked as a daily dose. This could affect compliance even with the weekly dose, which is delivered as a daily regimen, if daily dosing is too burdensome. 2) Concern over single dose. There may be a perception that 70 mg once weekly is a large amount of zinc at once, since the RDA for infants under 12 months is 5 mg of elemental zinc. However, Bates *et al.* supplemented young Gambian children from 0.5 to 2.3 years of age with 70 mg elemental zinc *twice* weekly over a 1.25 year period with no adverse effects(78). None are anticipated here. Nor is the total amount of zinc intake different between the two groups. Thus the data suggest that the risk is minimal, while the potential benefits would be substantial if the effect is the same between daily and weekly dosing. 3) Masking. Zinc is bitter. Flavouring will be added to disguise the taste. Infants in Group B may react to the taste in such a way as to provoke suspicion in the caretaker and healthworkers.

Specific Aim 4. *Rationale.* Studies have documented a preventive effect in children above 12 months of age, but not in younger infants, nor have studies been specifically designed to test

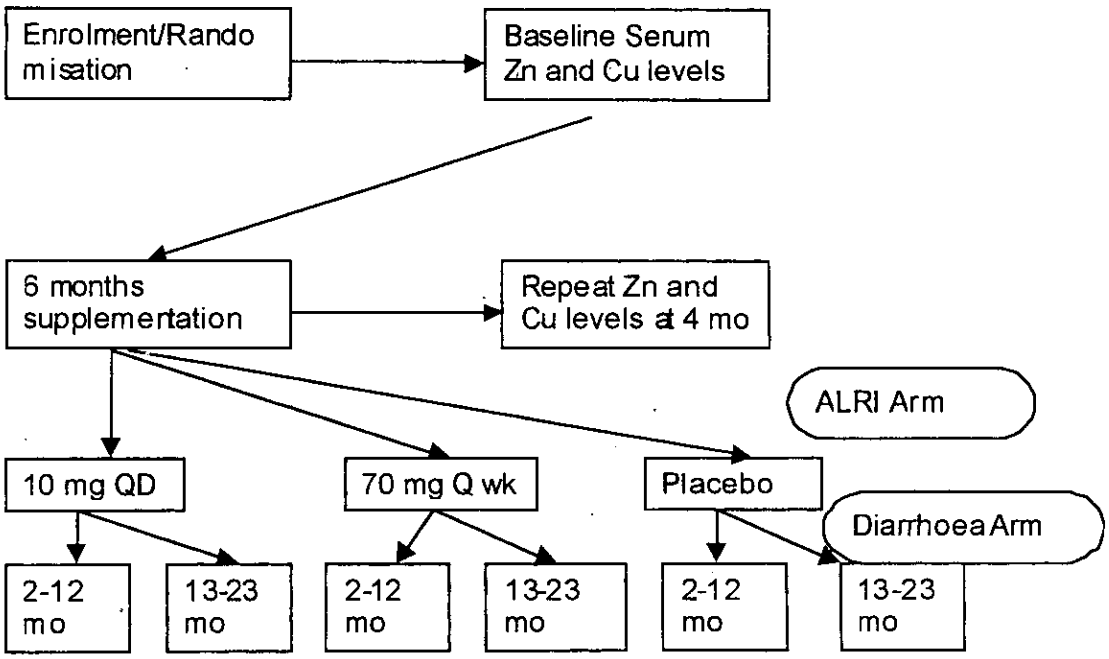
younger infants. *Protocol.* Infants will be post-stratified into two age groups in the analysis of the effect on diarrhoea; 2 to 11 months, 12 to 23 months. Stratified randomisation will not be used since the treatment groups are large(76). *Data.* Morbidity data will be analysed as outlined above. *Difficulties and Alternatives.* Minimal. Post-stratification will not affect randomisation, thus will not affect recruitment time or introduce bias. We took stratification into account in the sample size calculation, so power will not be diminished. However, gains in statistical precision will be small to nil if age is not related to outcome.

Secondary Aim 1. *Rationale.* To document whether or not improved isolated zinc status enhances growth in young infants. *Protocol.* Anthropometric measurements of weight and height will be collected at baseline and at monthly intervals on each infant (every fourth visit). Weight will be measured twice to the nearest 1 gram and height will be measured twice to the nearest 0.1 cm. The health worker will record the mean of both measurements. The anthropometric measurements will be numbered 0 - 6, beginning with the baseline measurement at the time of enrolment. The research assistant and the principal investigators will check to ensure that each patient has a total of seven measurements. *Data.* Growth: Height, weight. Calculated as z-scores for weight for age (WAZ), height for age (HAZ) and weight for height (WHZ). We will calculate geometric means and standard deviations by treatment group, and measure inter-group variation by ANOVA. *Difficulties and Alternatives.* Anthropometric measurements in the field are susceptible to imprecision if health workers are hurried. Second, inter-observer variation can affect overall growth outcome. Two countermeasures will be used. First, we will hire adequate numbers of staff so that a health worker sees less than 50 infants per day. Second, we will test and quantify the degree of inter-observer variation as part of the health worker training. A 10% sub-sample of measurements will be screened to monitor gross error. If variance remains high, a 20% sub-sample will be brought to clinic for anthropometry. These data will be used in the analysis.

Secondary Aim 2. *Rationale.* Infants with marginal copper status may be pushed into frank copper deficiency by an antagonistic divalent ion, such as zinc. Clinical signs of copper deficiency should be correlated with haematological parameters. *Protocol.* At baseline a 5 ml blood sample will be obtained for measurement of copper, haemoglobin and white blood cell count (WBC). Copper will be analysed by atomic absorption spectrophotometry. On the first visit after 120 days of supplementation, 5 ml of blood will be drawn from each patient and transported back to the Centre in ice. Serum will be analysed for copper. Serum copper levels will be correlated with clinical signs of copper deficiency, such as anaemia, leukopaenia, and neutropaenia, osteoporosis and pathological fractures resembling scurvy, as well as growth during data analysis. *Data.* Continuous numerical data will be summarised by treatment group, and the mean and standard deviations will be compared by ANOVA. These can be directly regressed on values for haemoglobin, WBC and neutrophil count. Transformation into categorical data will allow for modelling of high risk infants. *Difficulties and Alternatives.* 1) Sample collection. The community has a perception that health workers may collect blood for later sale to local hospitals for profit (private communication to the PI from community leaders). Caretakers have expressed distress about the discomfort caused to the infant during phlebotomy. Finally, prolonged transport times can affect the preservation of the serum samples. To circumvent these difficulties, we will bring infants to a field office for blood drawing. Blood samples will then be labelled and stored on ice for transportation later that day to the hospital laboratory. The cost of patient transportation, if needed, will be provided by the project. If drawing blood from the entire population is impracticable, a 20% sub-sample will be randomly selected and used for the analysis.

Secondary Aim 3. *Rationale.* Baseline zinc status is central to the hypothesis of a high prevalence of zinc deficiency. As this has been reported in previous studies, its demonstration is not a primary objective here. *Protocol.* Baseline and post 120 day supplementation blood samples will be drawn, as discussed above. *Data.* The data will be the same as for copper status, and will handled as discussed above. *Difficulties and Alternatives.* These will be the same as for copper, and will be handled in the manner discussed above.

Protocol summary. Figure 5.



Timeline. The entire study is projected to last for two years, as outlined below.

Figure 6.

Task	Time	In	Months						
Mapping	X								
Training	X								
Recruitment	X	X							
Zn Supplmnt		X	X	X	X				
Blood work		X			X				
Lab analysis						X			
Data entry		X	X	X	X	X	X		
Data analysis						X	X		
Writeup							X	X	
	3	6	9	12	15	18	21	24	

Methods

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Definitions

ALRI: Acute lower respiratory tract infection defined by respiratory rate (RR) alone has a low predictive value(42,69). In a recent community-based trial in India(42), when fever or lower chest indrawing was added to elevated respiratory rate, it increased the specificity from 58% to 82% and the positive predictive value (PPV) from 57.5% to 77.4%. We will use chest indrawing as described below. **A clinical exam with a finding of rales, rhonchi (or crepitations) is necessary for confirmation of diagnosis of severe or non-severe ALRI.** Therefore, every effort will be made to have caretakers notify the team or field office if a child has had an elevated respiratory rate and/or persistent cough for ≥ 24 hours.

Severe ALRI: History of cough for ≥ 24 hours with a RR > 50/min for infants < 12 months of age and RR > 40/min for infants 12 months of age or older with *any* of the following: nasal flaring, grunting, chest indrawing, central cyanosis, inability to feed, lethargy. No differentiation will be made between severe and very severe pneumonia in this trial, as this distinction will not affect treatment.

Non-severe ALRI: History of cough for ≥ 24 hours with a RR > 50/min for infants < 12 months of age and RR > 40/min for infants 12 months of age or older and *none* of findings for severe pneumonia. Since fever does not affect the intention to treat, it will not be measured in the field.

Episode of ALRI: will be defined as ≥ 1 day of cough with RR > 50/min for infants under 12 months of age and RR > 40/min for infants 12 months of age or older with abnormal auscultation findings (70). The duration of ALRI will be defined as the number of days for which age-specific elevated RR is reported. Unconfirmed reports of coughing, with or without tachypnoea, will be recorded and included in the analysis of the number of days with ALRI.

Episode of Diarrhoea will be defined as ≥ 1 day in which ≥ 3 stools of watery or loose consistency, or any loose stool with blood. Duration of diarrhoea will be defined as the number of days of reported loose stool.

Severe dehydration: will be defined according to the Dhaka method (See Appendix 3).

Seven or more symptom-free days (i.e., two consecutive visits) differentiate episodes of ALRI, and three or more symptom-free days (i.e., one visit) differentiate episodes of diarrhoea. Intention to treat, and the category of treatment recommended (outpatient or inpatient) will be recorded on the surveillance form, irrespective of whether or not the patient complies with the recommendation.

De jure population comprises the normal residents of a community, including those who may be absent during the enumeration.

De facto population consists of those who slept in the locality the night before the census was taken. Most national censuses record the *de facto* population, whereas in an intervention trial the *de jure* is the more appropriate(77).

Inclusion Criteria
Infants between 2 and 18 months, inclusively, at the time of enrolment, who comprise the *de jure* population of the target communities of Gulapbagh or Dholpur and whose parents give consent for their participation in the study.

Exclusion Criteria
Children with known reactive airways disease (i.e., have been placed on standing or PRN albuterol or other β2-agonist), tuberculosis, measles-related ALRI, severe malnutrition (> 2 SD below mean weight for height), persistent diarrhoea, hepatosplenomegaly, or who comprise the *de facto* community population.

Sample Size Calculation
Diarrhoea Range is 4.6 - 5.0 episodes/child/year for Dhaka urban incidence of diarrhoea(72). In order to detect a 20% reduction in the incidence of diarrhoea, using a SE of 2.5 and calculating 95% confidence and 90% power: we will need 132 infants per group. Allowing for 10% withdrawal gives 145/group for a total of 435 infants.

ALRI In a recent study of zinc supplementation in urban children 6 - 36 months old in the Subcontinent(42), *pneumonia incidence* among controls was calculated as 0.35 episodes/child/year. There are no recent published data on pneumonia incidence in this age group for Dhaka. The incidence of pneumonia in Matlab for the 2 month to 23 month age group is 0.3 episodes/child/year (Abdullah Baqui, personal communication). In order to detect a 30% decrease in the incidence of ALRI, (0.3 episodes/child/year), with a probability of a Type 1 error of 5% and a Type 2 error of 10% (in order to detect a difference between treatment groups) we will need 513 infants per group. Allowing for 10% withdrawal from the study, we will recruit 564 infants per group for a total population of 1,692.

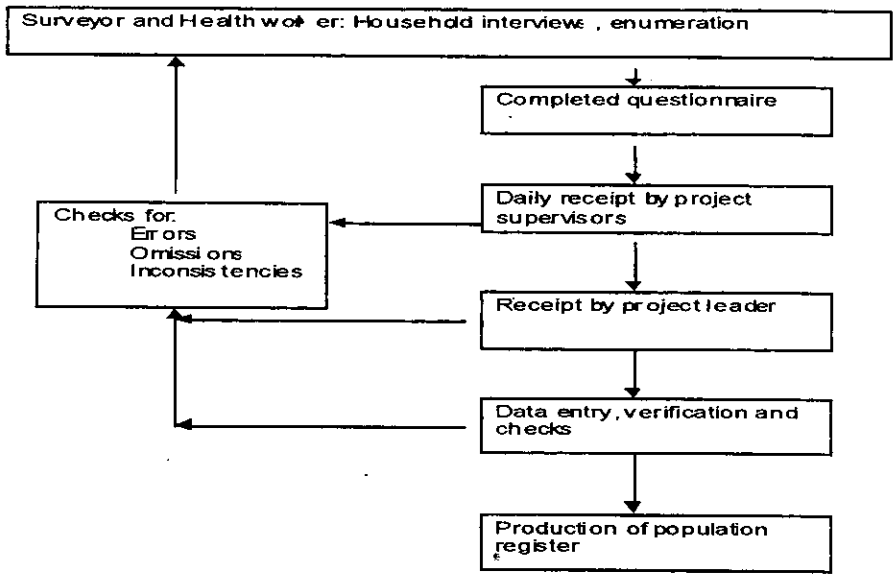
Population and Study Site Mapping
In order to find the requisite number of patients, assuming the under two year old population constitutes 4.5% of the total population, we would need a community of 2000/0.045 = 44,444 or about 45,000 pop in the community. A community has been identified to serve as the study site, Dholpur, which has recently served as an intervention site for a community-based education intervention trial, and its adjacent community, Gulapbagh. Both are urban slum areas in the south-western quarter of the city of Dhaka. Both have a population of 10,000 households, each with about 4 members per household. The under-five year population comprises about 20%. Taking the most conservative estimate of 4.5% for the under-two population as above allows us to utilise both areas for selecting treatment and placebo groups.

The area will need to be mapped. The average ground speed of a trained surveyor is 200 households/day in a densely populated urban area(77). Combined with other agenda, such as census taking, the rate may be halved. We will deploy 10 teams consisting of one

mapping assistant and one health worker. They will perform mapping and listing among 20,000 households in the study site. At a rate of 100 households per day, working five days per week, it will take 10 teams 4 weeks to complete the task.

There are well-trained staff and a field research officer available to the project familiar with that area and its inhabitants. These can be paired with contracted surveyors (masters' students from graduate schools in surveying programmes).

Flow chart for census and data collection (Adapted from Smith and Morrow).



Randomisation

Effectively all eligible infants in the study site will be selected, thus avoiding the need for random selection. Infants recruited will be randomised at the time of consent to one of the three treatment groups. This will be *fixed randomisation*. Allocation of study subjects will be uniform, as the assignment probabilities for test treatments and control groups are equal, according to the formula:

$$P1 = P2 = \ldots = Pi = \ldots = Pt+1$$

Where Pi, I = 1, ..., t, denote assignment probabilities for the t test treatment and Pt+1 denotes the assignment probability for the control group, and where $(t+1)\sum (i=1)\ Pi = 1$. Randomisation will be achieved by permuted blocks of variable length, where the smallest block size will be equal to the sum of integers of the allocation ratio, according to the formula $(t+1)\sum (i=1)\ ri = B$, where B is the minimum block size. As there are three treatment groups, B = 3. Thus, the range of block sizes will be 3 to 12. The larger the block the greater the likelihood of departure from the allocation ratio, so blocks greater than 4 times the number of treatment cells will be avoided. The block sizes used for the construction of the allocation schedule will not be divulged to the project personnel until after the trial to avoid unmasking.

Data Collection

Background personal, demographic and medical information; including identification (name, age, date of birth), birth history, previous hospitalisations or treatments for respiratory complaints, and feeding history; will be gathered using a standardised form at the time of enrolment (see APPENDIX 2). Trained staff members only will collect data using the standardised forms.

Clinical Management

Treatment of Severe Infection: Infants observed by the visiting nurse or the caretaker to have severe ALRI or diarrhoea with severe dehydration or bloody mucoid stools will be referred to ICDDR,B hospital for admission and treatment, according to existing hospital standards. Hospitalisation, treatment and transportation costs will be provided by the project.

Treatment of Non-severe Infection:

ALRI Infants with non-severe ALRI or diarrhoea will be treated in the community according to published WHO guidelines(70). This will be either Amoxycillin 40 mg/kg PO ÷ TID, or Cotrimoxazole 10 mg TMP PO ÷ BID. Both antibiotics will be continued for 5 days. Infants who fail to improve after 48 hours of antibiotics, or whose condition worsens on antibiotics in the judgement of the medical officer or the investigators will be referred to hospital for definitive care.

Diarrhoea Infants with diarrhoea and non-severe or no dehydration *and* no history of bloody mucoid stool will be offered ORS packets. Caretakers will be instructed to manage them according to published WHO guidelines. For "*Some* " dehydration this will be 50 – 100 ml after each loose stool, up to (approximately) 500 ml per day. The health team will reassess infants who require more for possible referral to hospital. If there is "*No sign*" of dehydration, caretakers will be advised to give extra fluid, continue feeding (particularly breastfeeding), and to notify the health team if any of the following develop: the infant starts drinking poorly or is unable to drink, becomes sicker, develops a fever, has blood in the stool or makes urine less than three times per day.

Medications for ALRI and dysentery, and ORS packets for diarrhoea will be provided by the project.

Anthropometry

Weights will obtained to the nearest 1g using an electronic digital scale standardised with a one kilogram standard weight each morning and prior to weighing each subject. Supine length measurements will be made with a metal sliding board. The mean of two consecutive measurements to the nearest 0.1 cm for all indices will be recorded as the observed value. Measurements will be compared to the standards of the National Center for Health Statistic (NCHS) data. Stunting will be defined as z score of < -2 for length or height for age (> 2 standard deviations below the median for the National Centre for Health Statistics reference population); and wasting as a z score of < -2 standard deviations for weight for length.

Laboratory Procedures

ICDDR,B Hospital, Dhaka, has a modern medical laboratory and routinely performs standard haematological tests, such as haemoglobin, haematocrit, white blood cell count and differential count according to internationally established methods. Samples will be collected in polypropylene tubes to avoid zinc contamination.

Zinc Assay. Samples (200 µl) in polypropylene tubes are diluted 1:12 with HNO3 and Briiz-35. Zinc is determined by flame atomic absorption spectrophotometry (AAS) using standard solutions prepared in the same matrix. AAS is equipped with nebulizer assembly (Shimadzu) and C2H2/air burner. Zinc hollow-cathode lamp is operated with background correction wavelength of 213 nm. Determination of zinc is done by aspiration of a series of working

standards (at least 4) and a standard curve is prepared for each lot by plotting the concentration (mg/l) versus absorbance using software provided by the manufacturer. Two quality control sera (low and high range) are run with each lot and if any value of these quality control samples is not within the acceptable range, the result is ignored and the whole lot is repeated.

Copper Assay. Samples (400 µl) in polypropylene tubes are diluted 1:12 with HNO3 and Briiz-35. Copper is determined by flame atomic absorption spectrophotometry using standard solutions prepared in the same matrix. Copper Again, a C2H2/air burner is used. AAS wavelength is 324.7 nm.

Quality Assurance

A 10% sample of the visits will be randomly selected and repeated by the research assistant or the investigators within 24 hours of the visiting health team. Periodic review and testing of field staff will be done and the degree of inter-observer variation will be quantified. The percentage of agreement and kappa statistic will be calculated to assess inter-observer variation. Spot checks at infants' homes will be made by the staff to measure the residual amount of zinc syrup in the bottles in order to assess compliance.

Consent Procedures

Consent will be obtained using a standardised form prior to enrolment in the study. Parents will be advised that their children's participation in this experimental study is voluntary and that they may withdraw their children from the study at any time, and that as new information becomes available that might affect this study, it will be made available to them. (SEE APPENDIX .)

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 hospital is an approximately 250 bed facility. Ill subjects who require hospitalisation will be brought back to hospital and treated in either the Short Stay Ward, a 120 bed facility that has an average stay of 20 hours, or the General Ward, a 60 bed facility. The existing hospital laboratory facilities will be used. The hospital laboratory processes approximately 18,000 serum samples per year for a wide range of tests. It possesses an atomic spectrophotometer that will be used to process our serum zinc and copper samples.

Data Analysis

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

X. Data analysis: SEE DESIGN ABOVE FOR DETAILS. *Recapitulation*: Type of data - continuous. Analysis type: Kaplan-Meier survival curve. Treatment and placebo group data will be compared by logrank test, to test the null hypothesis that the groups are the same with respect to frequency and duration of illness (and hospital stay). Hazard ratio will be performed to compare the relative frequencies and duration of illness times between groups. Modelling will be performed by Cox Regression. Categorical data will be analysed by chi square calculations (determine significant effects and trends). Effects will be tested with two-by-two table calculations (odds ratios). Modelling will be done by logistic regression (best-fit model for outcome and variables). Intergroup comparison of mean z-scores will be performed using one way analysis of variance (ANOVA) and determination of the F distribution. Normality will be verified by plotting the residuals.

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.

XI. Consent: SEE METHODS ABOVE.

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.

N/A

Literature Cited

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

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Dissemination and Use of Findings

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

~~The results of this research will be organised into standard scientific journal format and~~ submitted to peer reviewed journals for dissemination to the relevant academic and scientific communities.

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)**

N/A

Biography of the Investigators PLEASE SEE ATTACHED CV's

Voluntary Consent Form PLEASE SEE APPENDIX

Detailed Budget for New Proposal

Project Title: **Controlled Trial to Prevent Acute Lower Respiratory Tract Infection and Diarrhoea with Zinc Supplementation in Children Below 2 Years of Age**

Name of PI: **W. Abdullah Brooks, MD, MPH**

Budget Justifications

Please provide one page statement justifying the budgeted amount for each major item. Justify use of man power, major equipment, and laboratory services.

Budget Justification

- **ASG Faruqe**. Dr. Faruque has considerable experience in field epidemiology and in ALRI and diarrhoeal disease. He heads the hospital surveillance system. He adds experience and a necessary familiarity with the culture, in addition to the time he will provide, which will be equivalent to one half day per five-day workweek.
- **MA Wahed**. Mr. Wahed is a medical biochemist with over 20 years of experience. He will oversee and personally be involved with the processing of our serum zinc and copper samples, as well as ensuring the quality of adherence to the laboratory prctocol. Assuming that he devotes 3 hours/day of a five day work week to the processing of samples over a two month period, he will work 3/8 hours * 5 days / 7days * 21.5 days/month * 2 months / 258 days per year * 100 = **5% of the work year** during both years.
- **Research Investigator**. Since the PI will contribute no more than 50% time and the Co-PI 10%, we need a full-time project supervisor, qualified to oversee the entire project when no one else is available. This person must be experienced in the conduct of field studies, standardisation and quality assurance procedures, data collection methodologies, be able to conduct and supervise the clinical examination, and participate in the routine 10% sub-sample re-visits. Even when the PI is present, someone who understands both the aims and methods of the study, and who speaks the language, will be needed when there are difficulties. Since this is a large study, and the first field for the PI in Bangladesh, this investigator should be experienced and senior. At present there is one candidate with several years of field experience who has worked with other Centre personnel and has assisted in the background phase of this study.
- **Research Medical Officer**. A full-time physician in the field is essential to this project, as the determination of disease incidence is clinical. This individual must be accessible at the field office to accompany health workers to homes of children suspected to be ill, make an accurate diagnosis of the nature of the illness, assess the degree of severity and decide between outpatient management versus referral to hospital. The medical officer will participate in blood drawing. The medical officer will also spend at least 20% of their time in the field training the health workers to appropriately assess respiratory rate and dehydration, and will conduct periodic monitoring for quality assurance. Presently there are two candidates with field trial experience for the position.
- **Nurse**. Three GS5-level nurses will be needed particularly in the beginning to work independently and train and supervise the field research assistants to count respirations, assess dehydration, and perform anthropometric measurements. They will also conduct

home visits. Additionally, they will draw blood for serum zinc and copper both at the time of enrolment and after 120 days of supplementation. In order to complete the bloodwork during the first month of enrolment, we will need four full-time personnel. With 1700 infants, assuming a rate of 10 minutes per infant, it will take each person 42.5 man-hours. Distributing the work equally enables each to devote 10 hours per week, which will complete the work in 4.2 weeks or 30 days.

- **Field Research Assistants**. We will need 17 GS3-level field research assistants working 100% time to administer surveillance questionnaires and conduct exams (count respirations, perform monthly anthropometry). There will be about 850 infants per cycle visited twice weekly. Assuming a rate of 4 interviews per hour, an actual work day of 5 hours for interviews and a five day work week, each worker will interview 20 infants per day. We need to provide salary support for 17 interviewers over a 17 month period to complete the work, according to the formula:
- 1700 Interviews/7 days * 1 day/20 interviews * 1 worker/5 days/week * 7days/1 week = 17 workers.
- We will need them for 17 months accordingly: Training 1 month; Mapping 1 month; Recruitment 1 month/cycle X 2 cycles = 2 months; 6 month cycle of supplementation X 2 cycles = 12 months; mop up 1 month = 17 months.
- **Field Research Officer**. The study will require a research officer to oversee the work schedule of the research assistants, monitor the schedule for the monthly anthropometry, motivate the caretakers of the infants to comply with the provisions of the study (e.g., blood drawing, taking the supplements regularly, informing the staff of illness), ensure the coding of the questionnaire data, and the timely transport of data and materials (blood samples, questionnaires) to the Centre.
- **Secretarial**. This is a requirement of the Centre.
- **Data Entry Technician**. A full-time data entry technician is required. In order to ensure quality assurance and quality control, data must be checked, cleaned and entered from the beginning of the study. There will be 1700 intake forms, followed by 3400 surveillance forms per week. We do not project to recruit all of our infants at once, so the mean number of subjects is 850. Assuming a rate of 2 minutes per short form, data entry by a trained technician takes 57 hours per week for 1700 forms. The technician will need to be assisted at least one day a week to keep up with the workload.
- **Programmer**. In the second year, a statistical programmer will be required to assist with the running of our statistical analyses. This person will need to be familiar with SPSS and SAS software, and be able to make judgements about the appropriateness of statistical tests for a given type of data or question. We estimate that it will take about 40 hours to transform all of the data into appropriate format, followed by the equivalent of 35 hours per week for six to seven weeks for planning and conducting our analysis and reviewing our results.
- **Mapping Assistants**. We will deploy 10 teams consisting of one mapping assistant and one health worker. They will perform mapping and listing among 20,000 households in the study site. At a rate of 100 households per day, working five days per week, it will take 10 teams 4 weeks to complete the task.
- **Travel**. *International.* The PI will need to travel to the US the first year to meet with the international co-investigators and obtain consults from resident epidemiologists and biostatisticians. The second year he will need to travel to the US to present the findings of the study at Johns Hopkins (presentations are already being planned with different departments at the Schools of Public Health and Medicine). *Domestic.* We estimate a cost

of $3.00 per day per team of two for food and transportation, calculated against a 7-day workweek. There are 12 months of support in year 1 and 5 months in year 2.

- **Drugs**. The majority of the zinc must be prepared during the first year. This represents the estimated annual cost of preparation and packaging per dose per patient based on historical experience at the Centre for both years.
- **Non stock drugs**. *Year 1*: ALRI antibiotics. Assuming an infection rate of 0.3 episodes/child/year = 0.3 * 1700 infants * $5.00 Amoxycillin/infant * 9/12 year = $1,912.00 for Amox. Diarrhoea rate = 4 episodes/child/year = 4 * 1700 infants * $0.25 per ORS treatment (at $0.05/packet for 5 days) * 9/12 year = $1,275.00 for ORS. Luer Lock 3 cc Syringes 100/box at $10/box = $200. Set of 2000 Abbott 23 guage butterfly infusion needles 40/box at $33/box = $1700. Gloves 40 boxes of 100 at $4/box = $160. Sharps containers, 8 X-large 5 gallon large funnel set of 8 at $14/each = $112. B-D 3 cc sterile red-top vacutainer tubes 20 boxes of 100 at $18/box = $360. Total for year 1 = $5,719.00. *Year 2*: figures are the same, $5,719.00.
- **Office supplies**. Assuming we rent a field office with 800 square feet of floor space at $300/month for 12 months in year 1, we will spend $3,600 for rent and utilities. Capital costs for furniture: (2 desks, 15 chairs, 1 small fridge, lamps, 2 ceiling fans, 4 4-drawer file cabinets) = $1,500. Recurrent costs for paper, pencils, erasers, printer cartridges $50/month = $600. Total costs = $5,700. Year two is the same minus the capital costs. The figure is calculated for 5 months.
- **Serum copper**. The cost is the actual cost of the analysis to our lab, $3.00 per sample X 1700 samples.
- **Serum zinc**. The cost is the actual cost of the analysis to our lab, $3.00 per sample X 1700 samples.
- **Photocopying**. Intake questionnaires: $0.05/page * 3 pages/copy * 1700 copies = $255. Consent forms: $0.05/page * 2 pages/copy * 1700 copies = $170. Surveillance forms: $0.05/page * 1 page/copy * 2 copies/week * 52 weeks * 9/12yr = $6630/yr + 10% = $7,293. Training manuals: 0.05 * 10 pages/copy * 15 copies = $7.50. Total for first year is $7726.00. For year 2, minus the intake questionnaires and training manuals it is 0.05 * 1 page/copy * 2 copies/week * 52 weeks * 6/12 year = $4441.00.
- **Hospitalisation**. The Centre requires this. We assume ALRI infection rate of 0.3 episodes/child/year * 1700 infants * 0.33 admission rate * $6/day * 3 days * 9/12 year for year 1, and 6/12 year for year 2. For diarrhoea we assume a 10% rate for 1:4 episodes of diarrhoea at a rate of 4 episodes/child/year * $6/day * 3 days * 9/12year in year 1 and 6/12 in year 2. The figures are the respective sums.
- **Internet, fax, DHL, phone**. These are conservative estimates for the cost of these services/year of the study, taking into account overseas collaboration.
- **Library**. These reflect conservative estimates for the cost of retrieval and copying of articles, particularly in year 2 when we move towards publication.
- **Print and publication**. These are conservative estimates of the cost, which is present in year 2 only.
- **Miscellaneous**. This will cover sundry items, such as respiration counters from the GoB ARI Programme, glass dispensers and stainless steel spoons for sterile dispensing of zinc suspension in the field.
- **Indirect costs**. This is the limit of what the Swiss Development Corporation will pay to the Centre.

Other Support

Describe sources, amount, duration, and grant number of all other research funding currently granted to PI or under consideration. (DO NOT EXCEED ONE PAGE FOR EACH INVESTIGATOR)

NONE

Check List

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

4. Face Sheet Included ☐

5. Approval of the Division Director on Face Sheet ☐

- **Certification and Signature of PI on Face Sheet, #9 and #10** ☐

11. Table on Contents ☐

5. Project Summary ☐

- **Literature Cited** ☐

7. Biography of Investigators ☐

1. Ethical Assurance ☐

1. Consent Forms ☐

☐

10. Detailed Budget

Budget for 1 year, 1998 Name		Pay Level	No. of Staff	% Effort	Mo'ly Rate	Man/ month	Subtotal
Personnel							
3122	Dr. ASG Faruque	Co-PI	1	10%	1688	12	2,025
3122	MA Wahed	Co-Inv	1	5%	1400	12	840
3121	Research Investigator	No-B	1	100%	765	12	9,180
3121	Medical Officer	No-A	1	100%	632	12	7,584
3121	Nurse	GS5	3	100%	347	12	12,492
3121	Field Research Assistant	GS3	17	100%	224	12	45,696
3121	Field Research Officer	GS5	1	100%	347	12	4,164
3121	Mappers		10	100%	161	1	1,610
3121	Secretarial		1	20%	510	12	1,224
3121	Data Entry Technician	GS3	1	100%	224	12	2,688
3121	Health workers		4	100%	60	12	2,880
	Subtotal						**90,383**
Travel							
International							1,500
Domestic							10,950
	Subtotal						**12,450**
Supplies and Materials							
3701	Drugs (Zinc)						7,500
3712	Non-stock drugs						5,719
3704	Office supplies						5,700
3715	Length boards						200
	Subtotal						**19,119**
Lab. test and other							
4808	Serum Cu			$3*1700			5,100
4809	Serum Zinc			$3*1700			5,100
4806	Photocopying						7,726
4813	Hospitalisation costs						3,806
4817	Internet, Fax, DHL, Phone						500
4821	Library Service Charge						100
	Subtotal						**22,332**
Others							
4000	Training						100
4300	Print & Publication						500
4500	Misc						250
1 0300	Capital Exp/computer/printer		1		2500		2,500
2 0300	Capital Exp/weighing scales		4		200		800
	Subtotoal						**4,150**
	Total Direct						**148,433**
	Total Indirect (14%)						**20,781**
	Total Costs						**169,214**

Shamima Moin 3/6/98
Controller, Budget & Costing

Efficacy Zn Prevention ALRI/Diarrhoea < 2 y/o

Budget for 1 year, 1999 Name	Pay Level	No of Staff	% Effort	Mo'ly Rate	Man/ Month	Cost in US $
Personnel						
3122 Dr. ASG Faruque	Co-PI		10%	1756	12	2,107
3122 MA Wahed	Co-Inves		5%	1456	12	874
3121 Research Investigator	No-B	1	100%	796	12	9,552
3121 Medical Officer	No-A	1	100%	657	5	3,285
3121 Nurse	GS5	3	100%	361	5	5,415
3121 Field Research Assistant	GS3	17	100%	233	5	19,805
3121 Data analysis - Programmer X 2 mo		1	100%	832	2	1,664
3121 Field Research Officer	GS5	1	100%	361	5	1,805
3121 Secretarial		1	20%	530	12	1,272
3121 Health workers		4	100%	62	5	1,240
3121 Data Entry Technician	GS3	1	100%	233	6	1,398
Subtotal						**48,417**
Travel						
International						1,500
Domestic						4,560
Subtotal						**6,060**
Supplies and Materials						
3701 Drugs (Zinc)						5,000
3712 Non-stock drugs						5,719
3704 Office supplies						1,750
Subtotal						**12,469**
Lab. test and Other						
4808 Serum Cu		$3*1700				5,100
4809 Serum Zinc		$3*1700				5,100
4806 Photocopying						4,441
4813 Hospitalisation costs						2,537
4817 Internet, Fax, DHL, Phone						500
4821 Library Service Charge						150
Subtotal						**17,828**
Others						
4300 Print & Publication						500
4500 Misc						250
Subtotoal						**750**
Total Direct						**85,524**
Total Indirect (14%)						**11,973**
Total Costs						**97,497**

3/6/98

Shamima Moin
Controller, Budget & Costing

Controlled Trial to Prevent Acute Lower Respiratory Tract Infection and Diarrhoea with Zinc Supplementation in Children Below 2 Years of Age

Responses to Reviewers

Reviewer 1 (Mahalanabis)

1. The weekly dose has been changed to 70 mg QWk.
2. The intercurrent episodes of ALRI have been changed to 5 days (effectively two consecutive visits). Diarrhoeal episodes remain unchanged at 3 symptom-free days.
3. Double-blinding has been addressed in the design section, Specific Aim 3.
4. Surveillance is twice weekly.
5. Patients will be retained in the study, irrespective of compliance, so as not to bias the study.

Reviewer 2 (Ruel)

1. Design: the selection of subjects is changed and is now community-based.
2. The age-range for selection is 2 – 18 months.
3. The section on rationale has been modified and has been made included in the Design section and itemised by Specific-Aim (the format recommended by NIH reviewers, see
4. The statement "easy to administer" has been dropped. The adjective "inexpensive" is retained, however, as the reviewer's opinion is his own and does not reflect expert consensus. As routine administration of zinc is *not* yet policy, operational and distribution cost estimates would have to be based on clinical and field studies, which reflect a controlled (artificial) situation and *are* expensive. As the reviewer points out, zinc itself is inexpensive and distribution systems for mass consumption have not been developed.
5. The reviewer's comments are noted. In fact that study is cited as an example of one which did look at the age group of interest, but did not find an effect (Efficacy of Zinc as a Preventive Agent, paragraph 3).
6. Comments are included about the rationale for using zinc once weekly in both the Background (Efficacy of Zinc as a Preventive Agent, paragraph 6) and Design (Item 2 "Specific Aim 3" paragraph 1) sections. We could find no published studies done on a once weekly regimen, hence our proposal. We believe this to be an important piece of missing data in the growing literature of zinc supplementation as a preventive agent in children.
7. The layout of the proposal has been changed. The description of the study is in the Design section, which precedes Methods, where definitions and methodologies are given.
8. A discussion of the type of supplement, blinding etc is given in Design, Items 1 and 2. The actual amount (volume per dose) is under active discussion with the pharmaceutical company, but will be either 5 or 10 ml per dose.
9. The rationale for choosing partially breast-fed children was an attempt to standardise the diet, as most children in the age group above six months would be partially breast-fed. However, this has been dropped from the design. The type of diet will be one of the variables analysed, but is not a

specific aim of this study, as data on the protective effect of breastfeeding exist, thus diet did not factor into the sample size calculation.

10. The effect size was modified to fit the mean effect for ALRI based on meta-analyses (personal communication Robert E. Black, data not yet published). Sample size is driven by ALRI effect between treatment groups. Diarrhoea effect is 20%, which is more conservative than published effect sizes. Power is set at 90% for both, in order to detect treatment group differences.
11. The data analysis section has been changed and, as the reviewer appropriately observes, should be itemised by specific aim, which it is and is found in the Design section.

Reviewer 3 (Penny)

1. Hypothesis: The references to cellular immunity have been dropped and the wording modified to reflect measurement of clinical outcomes.
2. Background: A) The under-two year old population comprises the bulk of ALRI morbidity, and is worth studying. In order to have sufficient power to measure the effect of age on ALRI incidence within the same statistical parameters, we would have to recruit 3,386 infants. This would increase our cost from $263K to about $349K. Moreover, we would have to enlist an additional community in order to achieve the sample size. We believe that the cost would outweigh the benefits. However, we will stratify our analysis to examine the effect of age on diarrhoea incidence, which is an identified specific aim. B) The statement in "treatments" does not read "prevention is the only way to reduce morbidity", but "Prevention alone reduces the *incidence* of morbidity..." which is technically correct and reflects the PI's speciality training in preventive medicine. Perhaps the reviewer was not differentiating primary from secondary from tertiary prevention, which involve progressively increasing intervention by health providers. Indeed, primary prevention specifically reduces incidence, whereas the latter prevention methods involve effective case management and these primarily reduce disease prevalence and possibly mortality. (See Last J. Scope and Methods of Prevention in *Public Health and Preventive Medicine*. Appleton-Lange. 1996:3-10.) C) The wording of the proposal has been modified to emphasise that the intervention is correcting zinc deficiency and not that zinc is therapeutic, per se.
3. Zinc dosing. This section has been modified and does include a discussion of the benefits of cost and compliance for weekly dosing. The reference to toxicity states that it is "unlikely" in the doses being used, and all other references to toxicity have been dropped. The only toxicity issue remaining in the protocol has to do with the effect on copper status, and here haematological parameters i.e., serum Cu and leukopaenia, will be measured and correlated with clinical status.
4. Risks/benefits. A) We have made no claim that zinc reduces reactive airways disease (RAD). The benefits section reads, that supplements "may reduce the proportion of reactive airways disease that is *triggered by respiratory infection*..." which is a reasonable expectation. Studies have shown that a significant proportion of reactive airways disease is triggered by ALRI, particularly by respiratory syncytial virus (RSV) in young infants. (See Jeng MJ; Lemen RJ. Respiratory syncytial virus bronchiolitis. Am

Fam Physician 1997 Mar;55(4):1139-46.) If the incidence of ALRI is reduced, it stands to reason that the proportion of ALRI-mediated RAD exacerbations should also be reduced (see NIH Asthma Expert Panel Report 2, 1997). B) The reference to expense has been dropped.

5. Design. The design section has been significantly re-worked. The study is double blind.
6. Definitions. The field workers will visit twice weekly. Our definition of episode relies ultimately on clinical exam, not caretaker report of respiratory rate or cough, which has come under criticism for surveillance and reporting bias in other studies. Reports of illness will be included in the analysis of days with illness, as discussed in Methods. We believe the problems identified by the reviewer are correct, but are mitigated by twice weekly visits and corroboration of observed signs of respiratory distress with chest auscultation by a trained medical officer.
7. Anthropometry. Supine length is being measured, and the wording has been changed to emphasise this.
8. Laboratory. This section has been modified and indicates what precautions are being taken to avoid zinc contamination.
9. Randomisation. The study is double blind, and this is discussed in both Design and Methods. The individual bottles will have the child's code, not 'A', 'B', or 'C', which are instructions for the pharmaceutical company. The study does not rely on weekly recall. Visits are twice weekly but detection of disease (determination of incidence) is dependent on physical exam.
10. Compliance. The dose is 5 ml. Compliance will be measured twice weekly and on random spot checks (usually by the FRO and the investigators). The surveillance should be intense enough to discourage defaulting by caretakers who make a good-faith effort to comply. The only other option would be to have the field workers administer the syrup, which would be impractical for daily dosing in a study of this size. Indeed, it will provide useful insights if study subjects are non-compliant in significant proportion. We will not be able to assess the relative compliance between weekly vs daily doses, since the weekly dose will be masked as a daily dose. Children will not be withdrawn.
11. Treatment. Diagnosis of ALRI will neither depend on, nor include any references to, temperature since the presence or absence of temperature will not affect intention to treat. Numerous studies have shown fever to have too poor or inconsistent a correlation to warrant its use as a marker for ALRI, whereas chest indrawing, cyanosis, feeding, and abnormal auscultation findings consistently add to the predictive value of true ALRI when paired with tachypnoea (See Margolis et al.)
12. Inclusion criteria. The references to feeding status have been dropped.
13. Exclusion criteria. "Known" reactive airways disease has been defined. Rash was to be excluded to rule out measles, which increases the risk of subsequent infection. It has been dropped for concern over recall bias. The study site has not been used for other zinc trials. This was verified at a recent town meeting with community leaders, therefore we do not expect site contamination. An attempt will be made to limit the participants to one per household. If that is not possible, we will label the bottle for the mother to avoid confusion (e.g., a piece of tape with a circle vs an "X" to

distinguish two individuals). The actual dosing regimen will be same, irrespective of the true dose.

14. Quality assurance. Sample repeat visits are a valid method for assessing quality and variance of technique (see Smith P, Morrow R, Section 9.4 pp38), at least if the second party is blind to the findings of first. The reliability depends to an extent on the type and format of questions on the questionnaire. Ours will be categorical closed-type questions, which tend to show less variance than open questions. Additionally, we will conduct periodic reviews of interviewing technique, respiration and dehydration assessment among the staff.
15. Sample size. The effect size is 30% for both diseases, which is the mean effect noted from meta-analysis (Black, non-published data).
16. Data analysis. By "intention to treat", we presume this refers to compliance, which has been addressed above for both zinc supplementation and disease management.
17. Budget. The co-PI is presumed to work one-half day per five day workweek, or 10%.
18. The educational level of parents will be listed separately.
19. The finalised form of the questionnaire has not been completed.

Title: Controlled trial to prevent acute lower respiratory tract infection and diarrhoea with zinc supplementation in children below 2 years of age

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: Signature: [signature]

Date: 19/2/88

Position: Director

Institution: Society for Applied Studies

Detailed Comments

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

(Use additional pages if necessary)

Title:

PI:

Reviewer:

~~This research proposal is~~

~~In my opinion,~~

I give a very high priority to the topic of research in this proposal. The investigators are competent to do it and the institutional infrastructure is excellent. I have a few comments that follow.

Comments:

1. The weekly dose of 100mgm appears rather low. What is the justification for it?

2. For ALRI, is ~~three~~ three symptom free days enough to differentiate episodes?

3. How the double-blind status will be maintained when one group is getting a weekly dose? This should be described.

4. With once a week surveillance, how would they evaluate the respiratory rate and fever for attacks occuring between visits. The investigators should consider the feasibility of twice a week visit.

5. "Compliance check" (dropping rule). The patients with inadequate compliance should remain in the study and evaluated at the end of the study.

Title of proposal: Controlled Trial to Prevent Acute Lower Respiratory Tract Infection and Diarrhoea with Zinc Supplementation in Children Below 2 Years of Age

Autors: W. Abdullah Brooks, ASG Faruque, MA Wahed, George Fuchs

General Comments

The proposal is addressing an important question and the research has the potential to make an important contribution to the understanding of zinc deficiency and its consequences as well as its prevention in developing countries. The focus on children less than 2 years of age and on the two major causes of infant mortality in the developing world is extremely relevant.

My main concern with the proposal is the design, or more specifically the selection of subjects. The authors indicate that the study is a placebo controlled preventive trial in the community. It is not clear to me whether the authors are referring to a 'community trial'. In any case, the study proposes to estimate the community incidence of ALRI (p. 3). In order to do this, the sample would have to be selected in a truly random fashion to be representative of the population as a whole. Incidence rates would be estimated by following up this cohort of children over a pre-determined period of time. This is not what the study proposes to do. On the contrary, it proposes to select subjects only from children who are sick and who come to the health facility to be attended for diarrhea. Thus, the sample is not random and is likely to be seriously biased towards families who do attend facilities when their child is sick with diarrhea, and/or to children whose diarrhea is severe enough for parents to attend the facility. The proposed design is therefore inadequate to do any estimations of community prevalence or incidence of disease.

The recruitment of children with diarrhea also creates an age bias in the sample. Even within the first two years of age, the age pattern of diarrheal diseases tends to be different from that of pneumonia and ALRI. Although I do not know the case of urban Bangladesh in particular, I have seen in Latin America among primarily breast-fed children that the incidence of diarrhea tends to be low in the first 6-9 months of life, whereas mortality from ALRI is high, even in the first 3 months of life. Diarrhea peaks between 9-18 months of age. If the pattern is somewhat similar in Bangladesh, it would mean that the sample will be biased towards older children (9-18 months), rather than the 0-9 months old children who may be more at risk of ALRI, or at least increased risk of mortality from ALRI. For these reasons, and because the study is interested in both diarrhea and ALRI outcomes, the recruitment of children seen at a facility for diarrhea is inadequate.

A much better design for the proposed study would be to select a random sample of children from the community and to randomly assign them to the three treatment groups. I would also recommend to narrow down the age range to children less than 18 months of age so that the 6-month follow up would bring them to a maximum of 24 months, which is within the age range of

interest in this study. As indicated in the background section, 2/3 of all deaths due to pneumonia occur during infancy (the first year), so it would seem indicated to ensure that this age group is well represented.

Specific comments

Section on rationale (p. 5): This section is repeated further in the section on 'benefits of this project' (p. 9). The content is actually more appropriate for the section on benefits than for the section on rationale. The section on 'rationale' should include a discussion of why Bangladesh, why this age group, what makes us suspect that zinc deficiency is a problem in this population (e.g. diet, breast milk composition/quantity, complementary feeding, high rates of infection, etc.). In other words, why is this research justified in this particular context and what are the potential policy implications of the expected findings. Also, what is the rationale for the proposed treatment shedules (1/week vs 1/day).

Point 4 of the rationale: 'is inexpensive, easy to administer...': zinc supplementation, like iron supplementation, is not inexpensive from an operational and distributional point of view. The pills might be relatively cheap, but distribution systems that ensure high compliance have yet to be developed and are likely to be very costly.

Review of the literature (p. 7): the Ruel et al. study was done in children less than 2 years of age (age at recruitment was 6-9 months and the follow-up period was 7 months).

Some comments should be made about the rationale for using a once a week approach to treatment. Is there any literature, similar to that for iron, that would indicate that this could be equally effective as a daily dose and less likely to cause side effects?

Section on design (p. 10): Before giving the definitions and baseline data collection information, the description of the actual intervention should be given. One is interested in knowing what exactly will these children receive and how will the project be implemented before getting the other details.

The methods section should give more information on the type of supplement that will be provided: will a syrup-type supplement be used? What amount will be provided (total quantity of the supplement/day). Please provide information on preparation and also on how children who will receive the supplement once a week will be blinded to the intervention (i.e. will they be given a placebo 6 days a week and the supplement on one day to mimic the daily supplementation of the other group)? Please explain.

P. 13: why do children have to be partially breast-fed as opposed to exclusively? Couldn't they be exclusively breast-fed and just receive the supplement in addition?

Sample size: the magnitude of effects planned is large (45% for pneumonia incidence and 40% for

diarrhea). This means that the study will not have the power to detect differences of, say, 30 or 35%, which would still be biologically meaningful. Please provide information on sample sizes that would be needed for smaller effects to be detected (such as 30 or 35% for example).

The hypothesis of this study is that there will be no difference between the 2 treatments, but that both treatments will have a positive effect on morbidity reduction compared to the control group. When a study is interested in documenting the lack of difference between 2 treatments, sample size calculations are usually made based on a power of 90% as opposed to the usual 80%. This is because if you find no difference between the groups, you want to be sure that this is not because of lack of power, but rather, because there is really no difference between the groups (and in this case this is one of your main hypotheses).

The data analysis section (p. 14) should provide more details about which statistical analyses will be used to address each of the objectives of the study.

The budget for the study seems reasonable.

16/4

Shrestha

① Send copy to PI (A. Brooks)

② File original

Thanks,

SF.

Date: Tue, 14 Apr 1998 14:07:20
From: mpenny@iin.sld.pe
To: gfuchs@icddrb.org
Subject: protocol review

Dear george, I am sending this as a text file and as an annex, love, Mary

INSTITUTO DE INVESTIGACION NUTRICIONAL
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Telfs. 3496023- 3496024 - 3496144 - 3496145 - 3496150
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Apartado 18-0191, Lima 18 - Peru

13th April 1998

Dear George,

How nice to hear from you. I receive "Glimpse" regularly so I keep in contact with ICDDR,B's activities . You seem to be very busy and productive.

Thank you for sending the proposal "Controlled trial to prevent acute lower respiratory tract infection and diarrhoea with zinc supplementation in children below 2 years of age" for my review. I enclose my comments. I will try to send this by email as the quickest and cheapest route, assuming I can find your email address.

Regards to all my friends in ICDDR,B.

Best wishes,

Mary Penny
Medical Investigator
Instituto de Investigacion Nutricional

"Controlled trial to prevent acute lower respiratory tract infection and diarrhoea with zinc supplementation in children below 2 years of age".

General comment

This is an interesting and important study and in general the protocol has been well thought out. The issues addressed are important to both local and international child health. The principal investigator has plenty of experience in clinical research and of course the centre has considerable experience so that I am confidant that the study can be conducted as proposed. I support the project without qualification and my comments are intended to help ensure the success of the project.

If not already done, it would be worth discussing some of the aspects of the field work aspects with the investigators at ICDDR,B who have conducted similar community studies since it seems to me that details of the way that the supplement will be given and the collection of morbidity data are the weakest points in an otherwise

Specific comments:

Hypotheses

The first and second hypotheses relate to cellular immunity needed to resist infections but in fact the study is not designed to test cellular immunity and the study outcomes relate to morbidity. The investigators may be able to speculate that any changes they observe are attributable to improved cell mediated immunity but they will have no evidence for this. The hypotheses should be change to reflect the question to be answered by the project. The third hypothesis states that the preventive effect will be "dose-dependent". I think that this gives the idea that two different daily doses of zinc are being used, it would be clearer here and throughout the project (including the "Objective") to clarify that this is weekly versus daily administration of zinc. The Specific aims are quite clear except that you could add incidence and prevalence as this is the intention.

Background

The subject has been well reviewed and the emphasis on the fact that infant mortality is highest in the under two year olds is pertinent. As the investigators note, the highest mortality is in those aged under one year and there is less evidence on the benefit of zinc supplementation in this group. The discussion almost convinces me that a study in the under 1 years would really be the most interesting but I presume that there would not be sufficient children to allow this. However, it would be useful to stratify by under/over 12 months at enrollment and include this subgroup analysis in the analysis plan and if possible allow for this in the sample size calculations.

I do not entirely agree with the paragraph on "treatments ...." , the specific purpose of the mentioned treatments is not only to reduce mortality, and prevention is not the only way to reduce morbidity. There are lots of justifications for taking preventive measures as well as improving case management of illnesses, I don't think anyone would argue with the desirability of "prevention" regardless of the effectiveness of case management.

In order to put the potential benefit of the intervention into perpective I think it is important to mention that positive results of supplementation have been seen in zinc deficient populations, at the moment there is no evidence to suppose that zinc supplements will benefit children who are not zinc deficient, in other words we are talking about correcting a deficiency rather than a therapuetic or pharmacological effect. This does not weaken the potential importance of the intervention as zinc deficiency is likely to be common in nearly all populations with high infant morbidity and mortality. I think these issues should be mentioned in the proposal. If the investigator wants to argue for a pharmacological effect of zinc this could be discussed.

Zinc dosing

The investigators propose to compare once weekly with daily doses of zinc. Presumably since it is known that the total dose with the different they could have achieved this by giving a lower daily dose in comparison with 10mg daily. There has been considerable discussion about the potential difficulty of having to give a daily zinc supplement and there is interest in weekly dosing since if this worked it might be easier to administer, possibly increase compliance and perhaps more importantly indicate that if , in practice, many daily doses were forgotten the intervention might still be efficacious. There are also cost considerations. Weekly dosing would reduce the cost of this potentially expensive intervention. I think these logistics issues are the real reason for testing weekly versus daily rather than the fear of toxicity and this should be discussed.

...cacious. There are also cost considerations. Weekly dosing would reduce the cost of this potentially expensive intervention. I think these logistics issues are the real reason for testing weekly versus daily rather than the fear of toxicity and this should be discussed.

I do not agree that a larger preventive effect of the weekly supplementation would necessarily suggest that the daily doses were toxic.

Risks and benefits

I think that there is not enough evidence to suggest that the zinc supplements will reduce reactive airways disease, the promise of reductions in pneumonia are sufficient to make this an attractive intervention.

I don't think that this intervention could be regarded as "inexpensive", it will not be compared with therapeutic treatments but with public health preventive measures. The investigator might explain how this intervention will fit in with IMCI.

Research design and methods

The design would be greatly strengthened if the study was double blind, as I understand it the weekly dose group will be distinguishable, could you consider giving everyone daily doses with the weekly doses group 6 placebos to one zinc dose?

Definitions

No problem with diarrhea. The fieldworker will only visit once a week but the definition of ALRI requires raised RR and one other sign or symptom of which fever >=37.9, chest indawing and cyanosis which presumably depend on the fieldworker observation. If you are not visiting daily how will you be able to measure duration of the episode and how will you define the end of the episode in order to differentiate episodes? If the mother says that the child had fast breathing , cough, fever 6 days ago but is normal on the fieldworker exam this episode would be missed. This is why many community studies end up defining episodes of ALRI as cough with raised respiratory rate (+/- other symptoms) at any time during the episode and do not attempt to define duration except in rather imprecise ways. Instead they use prevalence of symptoms which the mother can reliably report, i.e. cough. This aspect does need a bit more thought.

Anthropometry

At this age you will measure supine length rather than height.

Laboratory investigations

The precautions to be taken to avoid zinc contamination should be explained, especially as the blood samples are to be collected in the homes.

Randomization

It is not clear to me if it is really double-blind or whether the weekly and daily dosing schedules will be evident. What dosing regime will the placebo group follow? It seems that the supplements will be divided into "A", "B", and "C". This makes it very easy for the codes to be broken especially as the study will be doing laboratory analyses. The study would be much stronger if the medicine bottles could be individually relabelled with the code of the child so that the danger that fieldstaff and investigators can know which

[redacted]nity follow-up.

It seems a pity to rely on weekly recall data when we know that after 48 hours the reliability of recall goes down. If I remember correctly investigators at ICDDR,B are among those who have demonstrated this. Could you not do twice weekly visits and only accept recall over the preceding week? If you visit once a week and the mother is not present then more than 7 days will pass before the next visit and recall for over this period really should not be considered reliable and you will then have gaps in your information about the child. If you schedule visits twice a week if the mother is absent for one visit you still have a chance of recuperating the information at the next visit.

Compliance

This is a weak aspect of the study and needs more discussion of practical details. How big is the daily dose (5ml?) how will the residual amount be measured, with what precision? How will you know whether the mother has given the dose daily or if she realizes she has forgotten a dose she may give a larger dose so that the residual amount will be the same? This is always a problem when dosing cannot be observed and does weaken the study since if you have negative results your only evidence that this is not due to non compliance will be the biochemical results. At the very least you could make additional spot checks between the healthworker visits in order to double check the residual in the bottle. These issues should be discussed more fully in the proposal. If a mother is not compliant but wants to remain in the study to "remind her of the study agreement" seems to be to be an invitation to make sure that she appears to be compliant next time by ensuring that the medicine has disappeared even though she may not necessarily have given it to the child. Children who are not compliant should not be withdrawn from the study as this may well introduce a bias, mothers of children who do not seem to be responding because they have more illness, for instance, may be less compliant and would end up being withdrawn. If the children are not followed up until the end you will not be able to do intention to treat analysis. These issues are especially important if there is a possibility that the codes may be inadvertantly broken. You could set a priori definitions for compliance and apply these in your analysis.

Treatment for non-severe infection.

Will you only take temperature if the child has raised RR?

Inclusion criteria

Why only include partially breastfed children? How will this be defined? What effect will this inclusion criterion have on the number of children available and how will this effect the age distribution.?

Exclusion criteria

In these very young children how will you define reactive airways disease? Why exclude children with rash? What sort of rash? How and who will make the individual decisions on rashes? Mention the justification for excluding exclusively breast or bottle fed babies. I imagine that children who have previously received supplements of zinc, including children who have already participated in the study, will be excluded. What will the policy be for children who live in the same household as a study participant, for instance siblings or cousins? . In these cases there is the risk of confusion between the medicines.

Quality assurance

I am not sure how useful repeat visits are to calculate and assess

quality assurance

I am not sure how useful repeat visits are to calculate and assess inter-observer variation. Mothers may well change their report especially when asked to report over a long period and an exam conducted up to 24 hours after the first visit may be completely different without being inaccurate. Since RR are such an important study outcome it would be better to do some simultaneous visits with both supervisor/research assistant/investigator and health worker counting the RR at the same time. Regular standardization exercises against a "gold standard" are also helpful and the results can be reported.

Outcomes - see earlier discussion

Sample size
Considering that not all studies have found an effect on ALRI incidence, a 45% reduction may be optimistic in the sample size calculation. A 40% reduction in diarrhea also seems high but as usual these figures may be dictated more by the budget than other considerations.

Data analysis
Mention whether intention to treat or not, if not explain selection.

Budget
Under manpower the co-principal is said to contribute one hour per week, a imagine this is one day per week as in the budget calculation!

The budget seems reasonable, although it is difficult to know without inside knowledge on how many visits the field team can be expected to do per day and how many repeat visits they would have to make.

Appendices

Appendix 2
II D. Ask education level of mother and father separately

Appendix 3
III How will the form be used to determine duration of an episode. For instance a child who is reported to have had two days of diarrhea in the last week might have had these two days at the beginning of the week and be symptomfree for three days or may have started two days ago and so the episode might be going to last longer. Under G you mention diarrhea but the definition to be used is Three or more liquid stools so the question should be asked in terms of "how many stools did the child have each day" and "how many of these were loos or watery" ?

I hope that these comments are useful, good luck with the study!

ALRI/Diarrhoea Prevention with Zinc Trial in Infants < 2 Year Old Consent Form

1. **The activity involves an experiment.** We are asking you to allow your child to participate in an experiment. We want to know if zinc can prevent pneumonia and diarrhoea in infants. To find out, we will give some infants syrup with zinc and others syrup without zinc for six months. We will come to your home twice a week to ask if your child has been sick since our last visit. Once a month, we will meausre your child's height and weight.

2. **The scope, aims and purpose of the research.** Pneumonia and diarrhoea cause much of the sickness and death in young infants. We have medicine to treat sick infants, but we want to prevent them from becoming sick. We know that zinc is important for health. Most children in Bangladesh do not get enough zinc in their food. We want to know if we can prevent children from getting pneumonia and diarrhoea by giving them zinc.

3. **Foreseeable harm and possible level of risk.** There have been many other studies in which children have been given zinc, and no children have been harmed by the doses we will use. A few children get a mild stomach ache that lasts a short time. Most do not get any stomach ache. We need a small sample of blood before your child starts taking the syrup, and again after 120 days. We will clean the skin and use a sterile needle to guard against infection.

4. **Likely or expected benefits.** Your child may have fewer episodes of pneumonia and diarrhoea by taking zinc. We will also check to see if zinc makes your child grow taller.

5. **Alternative procedures, treatments or preventive strategies.** Other than immunisations, including the ones that all infants should receive – including infants in this study - there are no treatments to prevent pneumonia and diarrhoea.

6. **Subjects will be notified of new information that might affect their participation.** We will be closely watching the progress of all the children in our study. We will notify you if we detect any problems, or if we get new information that gives us the answers we are looking for.

7. **Confidentiality will be maintained.** All the information you give us will be kept in a locked cabinet. Only our staff will have access to it. We will not give it to anyone.

8. **Explanation about medical treatment and care for research-related illness.** If your child gets sick with pneumonia or diarrhoea during this six month period, we want you to notify us immediately so that we can give you medicine. Your infant should continue taking the syrup, unless we advise you to stop. If your child is very

ill with pneumonia or diarrhoea during this six months, we will send you to ICDDR,B Hospital, where we will provide care.

9. **Participation is voluntary and refusal to participate will involve no penalty.** You may withdraw your child from this study at any time, and there will be no penalty. If you have any questions, please ask us.

_____(Parent) _____(Witness) __/__/__ (d/m/y)

আন্তর্জাতিক উদরাময় গবেষণা কেন্দ্র
মহাখালী, ঢাকা-১২১২

সম্মতি পত্র

জিংক প্রয়োগের মাধ্যমে ২ বছরের কম বয়সী শিশুদের নিউমোনিয়া ও ডায়রিয়া প্রতিরোধ

আপনার শিশুকে এই গবেষণায় অংশগ্রহণের অনুমতি দেয়ার জন্য আপনাকে অনুরোধ করা হচ্ছে। এই গবেষণা থেকে আমরা জানতে চাই জিংক শিশুদের ডায়রিয়া ও নিউমোনিয়া প্রতিরোধ করতে পারে কি না। এই জন্য আমরা কিছু সংখ্যক শিশুকে জিংক সহ সিরাপ এবং কিছু সংখ্যক শিশুকে জিংক ছাড়া সিরাপ ৬ মাস পর্যন্ত খাওয়াবো। আমরা সপ্তাহে ২ দিন আপনার বাড়ীতে আসবো এবং শেষবার দেখা হওয়ার পর আপনার শিশু অসুস্থ হয়েছিল কিনা তা জানতে চাইবো। আমরা প্রতিমাসে ১ বার আপনার শিশুর উচ্চতা ও ওজন পরিমাপ করবো।

বেশীর ভাগ শিশু নিউমোনিয়া ও ডায়রিয়ার জন্য অসুস্থ হয় ও মারা যায়। শিশুরা অসুস্থ হলে আমরা ওষুধ দিয়ে চিকিৎসা করি কিন্তু আমরা শিশুর অসুস্থতাকে প্রতিরোধ করতে চাই। আমরা জানি স্বাস্থ্যের জন্য জিংক গুরুত্বপূর্ণ। কিন্তু বাংলাদেশের বেশীর ভাগ শিশু খাদ্যের সঙ্গে যথেষ্ঠ পরিমাণে জিংক পায় না। আমরা জানতে চাই জিংক খাওয়ানোর মাধ্যমে শিশুদের নিউমোনিয়া ও ডায়রিয়া থেকে রক্ষা করা যায় কিনা।

বিভিন্ন গবেষণায় শিশুদের জিংক প্রয়োগ করে দেখা গেছে যে আমরা এই গবেষণায় শিশুদের যে পরিমাণ জিংক খাওয়াবো তা শিশুর কোন ক্ষতিসাধন করবে না। আপনার শিশুকে জিংক খাওয়ানোর আগে ১ বার এবং জিংক খাওয়ানোর ১২০ দিন পর ১ বার রক্ত পরীক্ষা করে দেখা হবে। রক্ত নেয়ার সময় শিশুকে রোগ সংক্রমণ (ইনফেকশন) থেকে রক্ষা করার জন্য শিশুর ত্বক পরিস্কার করে নেয়া হবে এবং জীবাণুযুক্ত সূঁচের মাধ্যমে রক্ত সংগ্রহ করা হবে।

জিংক খাওয়ানোর ফলে আপনার শিশুর ডায়রিয়া ও নিউমোনিয়া রোগের প্রকোপ কমে যাবে। জিংক খাওয়ানোর ফলে আপনার শিশু আরও বাড়ছে কিনা (উচ্চতা ও ওজন) তাও পরীক্ষা করে দেখা হবে।

৬টি মারাত্মক রোগের টীকা দেয়া ছাড়া আর অন্য কোন উপায়ে নিউমোনিয়া ও ডায়রিয়া রোগ থেকে শিশুকে রক্ষা করা যাচ্ছে না এবং গবেষণায় অংশগ্রহণ কারী শিশুসহ সব শিশুদেরই এই টীকা নেয়া উচিৎ।

আমরা খুব সতর্কতার সাথে গবেষণায় অংশগ্রহণকারী প্রতিটি শিশুকে পর্যবেক্ষণ করবো। গবেষণা চলাকালে যদি কোন সমস্যা দেখা দেয় অথবা আমরা নতুন কোন তথ্য পাই, যার জন্য এই গবেষণা করা হচ্ছে, তবে তা আপনাকে জানানো হবে।

আপনার দেয়া সকল তথ্য একটি তালাবদ্ধ কক্ষে রাখা হবে। শুধুমাত্র গবেষণাকর্মীরাই এইসব তথ্য দেখার সুযোগ পাবে। এই তথ্য অন্য কাউকে দেখার সুযোগ দেয়া হবে না।

গবেষণা শুরু করার ৬ মাসের ভেতরে যদি আপনার শিশু নিউমোনিয়া ও ডায়রিয়া দ্বারা আক্রান্ত হয় তবে সাথে সাথে আমাদের জানাবেন যাতে আমরা আপনার শিশুকে ওষুধ দিয়ে চিকিৎসা করতে পারি। আমরা নিষেধ না করা পর্যন্ত আপনি শিশুকে জিংক খাওয়ানো বন্ধ করবেন না। যদি আপনার শিশু নিউমোনিয়া বা ডায়রিয়া দ্বারা মারাত্মকভাবে অসুস্থ হয়ে পড়ে তবে আই,সি,ডি,ডি, আর, বি (মহাখালী কলেরা) হাসপাতালে তার চিকিৎসা করা হবে।

এই গবেষণায় আপনার শিশুর অংশগ্রহণ সম্পূর্ণভাবে আপনার ইচ্ছার উপর নির্ভর করে। আপনি চাইলে যে কোন সময়ে গবেষণায় অংশগ্রহণ থেকে আপনার শিশুকে বিরত রাখতে পারবেন। তার জন্য আপনাকে কোন ক্ষতিপূরণ দিতে হবে না। আপনার কোন প্রশ্ন থাকলে অনুগ্রহ করে আমাদের জিজ্ঞাসা করুন।

মাতা/পিতা/অভিভাবক	তারিখ	গবেষকের স্বাক্ষর	তারিখ

Prevention of ALRI and Diarrhoea with Zinc Community Trial
Intake Questionnaire

1. Identifying Information
   - 1.1. Date
   - 1.2. Name
   - 1.3. Patient record number
   - 1.4. Age
   - 1.5. DOB
   - 1.6. Sex

2. Sociodemographic Information
   - 2.1. Residence address
   - 2.2. Age of parents
   - 2.3. Occupation of parents
   - 2.4. Mother working outside home
   - 2.5. Education of parents
   - 2.6. Primary caretaker for child
   - 2.7. Number of children under age 5 y/o besides patient
   - 2.8. Number of children sleeping same room
   - 2.9. Latrine t ype
   - 2.10. Animals (type, location kept)

3. PMH
   - 3.1. Birth Hx (location, FT, BW, Preg/deliv problems)
   - 3.2. Feeding Hx
   - 3.3. RAD
   - 3.4. Measles, rashes
   - 3.5. Hospitalisations
   - 3.6. Meds
   - 3.7. Allergies
   - 3.8. Immunisations
   - 3.9. Total, timing
   - 3.10. UTD (Y/N)

4. PE
   - 4.1. By Systems, including:
   - 4.2. Skin (rash?)
   - 4.3. RR, GFR, Auscultation

5. Anthropometry
   - 5.1. Height (0.1 cm)
   - 5.2. Weight (1 gm)

6. Blood sample taken
   - 6.1. Zinc

6.2. Cu
6.3. CBC

Prevention of ALRI and Diarrhoea with Zinc Community Trial
Field Questionnaire

1. Identifying Information
   - 1.1. Date of enrolment
   - 1.2. Date of visitation
   - 1.3. Visitation number (circle 1 - 25)
   - 1.4. Interviewer name
   - 1.5. Interviewer code
   - 1.6. Name of subject
   - 1.7. Patient record number
   - 1.8. Randomisation number
   - 1.9. Age
   - 1.10. DOB
   - 1.11. Sex
   - 1.12. Relation of caretaker (interviewee) to subject

2. Compliance with supplementation
   - 2.1. Schedule of administration (QD vs QWk)
   - 2.2. Caretaker report compliance (Y/N)
   - 2.3. Medicine bottle present?
   - 2.4. Quantity administered 7 days ago (mL)
   - 2.5. Quantity remaining today, difference (mL)
   - 2.6. Quantity administered today (mL)

3. Surveillance data - Recall
   - 3.1. Cough during past week (Y/N)
   - 3.2. Duration (days)
   - 3.3. Tachypnoea during past week (Y/N)
   - 3.4. Duration (days)
   - 3.5. Chest indrawing during past week (Y/N)
   - 3.6. Duration (days)
   - 3.7. Feeding difficulties (Y/N)
   - 3.8. Duration (days)
   - 3.9. Convulsions (Y/N)
   - 3.10. Duration (days)
   - 3.11. Fever during past week (Y/N)
   - 3.12. Duration (days)
   - 3.13. Diarrhoea during past week (Y/N)
   - 3.14. Duration (days)
   - 3.15. Seen by doctor past week (Y/N)
   - 3.16. Reason (categories)
   - 3.17. Visit hospital past week (Y/N)
   - 3.18. Reason (categories)

4. Surveillance data - Observed

- 4.1. Cough present (Y/N)
- 4.2. Duration (days)
- 4.3. Tachypnoea present (Y/N)
- 4.4. Duration (days)
- 4.5. Rate (R/min)
- 4.6. Chest indrawing (Y/N)
- 4.7. Duration (days)
- 4.8. Fever present (Y/N)
- 4.9. Value (° C)
- 4.10. Diarrhoea present (Y/N)
- 4.11. Duration (days)
- 4.12. Quality (watery/bloody-mucoid)
- 4.13. Frequency
- 4.14. Dehydration present (Y/N)
- 4.15. Some/Severe

5. Treatment (Y/N)
   - 5.1. Amox
   - 5.2. Other Abx
   - 5.3. ORS (No. of packets)

6. Referred to hospital (Y/N)

7. Anthropometric data (circle visits 4,8,12,16,20,24)
   - 7.1. Height (0.1cm)
   - 7.2. Weight (1gm)
   - 7.3. HAZ
   - 7.4. WAZ
   - 7.5. WHZ

8. Blood work [visit 34+] (Y/N)

CURRICULUM VITAE

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Education

1994-Present Baltimore,	Johns Hopkins School of Hygiene and Public Health, MD Preventive Medicine Residency
1994-1995 Baltimore,	Johns Hopkins School of Hygiene and Public Health, MD Master of Public Health (MPH)
1991-1994	The New York Hospital/Cornell Medical Ctr. New York, NY Residency - Pediatrics
1985-1991	Stanford University School of Medicine, Stanford CA Medical Doctorate (MD)
1983-85	American River College Sacramento, CA Post-baccalaureate sciences
1976-1978	University of California, Los Angeles Bachelor of Arts (BA) International Relations
1974-1976	University of California, San Diego Political Science

Research Activities

July-October 1995 Bangladesh	International Centre for Diarrhoeal Disease Research, (ICDDR,B) Dhaka, Bangladesh Multicentre Study on Lower Osmolar oral rehydration solution (ORS). Clinical trial to assess the efficacy of lower osmolarity ORS relative to standard ORS (WHO formula) in adults with cholera and infants under two years of age with acute watery diarrhoea.
July-October 1995	ICDDR,B Dhaka, Bangladesh Analysis of surveillance data on risk factors for Amoebic dysentery among patients hospitalised and clinic patients at the Diarrhoea Hospital.
July-October 1995	ICDDR,B Dhaka, Bangladesh Analysis of surveillance data on risk factors for Cryptosporidiosis among hospitalised and clinic patients at the

	Diarrhoea Hospital.
July-October 1992	University Hospital, Salvador Bahia, Brazil. Field study on risk factors for invasive diarrhoeal disease among slum children in Salvador. Included collection of epidemiological data as well as isolation of pathogens and testing with DNA probes.
Oct-December 1990	The Center for Disease Control and Prevention Atlanta, GA Epidemiology Intelligence Service Clerkship for Medical Students - Enteric Branch. Included outbreak investigation of E. coli 0157-H7 in Helena MT among children at an elementary school, and surveillance system analysis for Salmonella typhi carriers among state health departments in all 50 states and territories.
July-Sept 1986	The National Institutes of Health (NIH) Bethesda, MD Division of Parasitology. Isolation of Leishmania major attachment proteins to the macrophage, as a possible means of prevention of parasitic infection.
1985-1986	Stanford University School of Medicine Stanford, CA Identification of heat-shock proteins in Fasciola hepatica morphogenesis, as a model for morphological regulation in Schistosoma species.

Professional

1996-1997	Chief Resident, Johns Hopkins Preventive Medicine Pgm Baltimore, MD
1996	Consultant for PAHO, EPI/SVI Georgetown, Guyana Planned and implemented mass vaccination campaign against measles for all children aged 12 - 59 months
1996	Consultant for Consultants in Epidemiology and Occupational Health Washington, DC Principal investigator in risk factor analysis for Non-Hodgkins Lymphoma
1995+	St. Joseph's Hospital Pediatric Express Baltimore, MD Emergency room physician for pediatric patients

1995+	Sinai Hospital Emergency Room Baltimore, MD Emergency room physician for pediatric patients
1995+	Baltimore County Health Department Baltimore, MD Physician for School-based clinics in Baltimore County School District
1994+	Kennedy Krieger Institute Baltimore, MD Pediatric physician for inpatients

Grants

1996 in S,	Small study on serological-based evaluation of silicate exposure patients with scleroderma: NIH; Lawless D, Brooks W, Lamm Teed K. Not Funded.

Presentations

April 1993 results on children in	Department of Pediatrics Grand Rounds: Original research risk factors for invasive diarrhoeal disease among slum Salvador, Bahia, Brazil The New York Hospital/Cornell Medical Ctr, NY, NY
Oct 1990 the	Genetic evidence of the common origin and essential oneness of human race: Association for Bahá'í Studies, Atlanta, GA

Awards and Honours

Jul-Oct 1995 Disease Division	International Fellow, The International Centre for Diarrhoeal Research, Bangladesh (ICDDR,B) Clinical Sciences Dhaka, Bangladesh
1994-1995 Public Baltimore,	Health and Child Survival Fellows Scholarship for Master of Health degree Johns Hopkins School of Hygiene and Public Health, MD
1985-1986	Medsholars Award for independent research Stanford University School of Medicine, Stanford, CA
1986 research	Ciba-Geigy Summer Internship Award for medical student National Institutes of Health, Bethesda MD
1974-1975 Academic	Pacific Palisades Chamber of Commerce Scholarship for Achievement and Student Leadership

Teaching Experiences

1995	Johns Hopkins University Intersession Course on Career Options in Public Health
1993	Methodist Community Hospital, Brooklyn NY Chief Resident: Clinical training of pediatric residents as part of that hospital's accreditation process

Memberships and Activities

1994+	American Public Health Association
1993+	American Academy of Pediatrics
1995+	Association of Preventive Medicine Residents
1992+	American Academy for the Advancement of Science
1990+	Bahá'í International Health Agency
1977+	Member of Bahá'í Faith, Bahá'í Community of the United States of America

Professional Licensure and Certification

Board Certified, New York Board of Medical Examiners

Board Certified, Maryland Board of Medical Examiners

Certified, American Heart Association
Basic Cardiac Life Support (BCLS)
Advanced Cardiac Life Support (ACLS)
Pediatric Advanced Life Support (PALS)

Languages

French - Speaking, reading, writing proficiency

Portuguese - Speaking, reading, writing proficiency

Arabic - Reading, writing comprehension (with difficulty)

Swahili - Conversant (with difficulty)