Attachment 1.

FACE SHEET) ETHICAL REVIEW COMMITTEE, ICDDR,B. Date 1/6/96

Principal Investigator Rashidul Haque Trainee Investigator (if any)

Application No. 96-010 Supporting Agency (if Non-ICDDR,B) [illegible]

Title of Study Field trial of beta-carotene and anti-helminthic therapy to improve

Project status:
(✓) 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).

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

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)

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

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

5. Will signed consent form be required:
(a) From subjects — Yes (No)
(b) From parent or guardian (if subjects are minors) — (Yes) No

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

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

* If the final instrument is not completed prior to review, the following information should be included in the abstract summary:
1. A description of the areas to be covered in the questionnaire or interview which could be considered either sensitive, or which would constitute an invasion of privacy.
2. Examples of the type of specific questions to be asked in the sensitive areas.
3. An indication as to when the questionnaire will be presented to the Cttee. for review.

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

Rashidul Haque
Principal Investigator

Trainee

ENTERED 2 1 JUN 1996

CHECK-LIST FOR SUBMISSION OF PROPOSALS TO THE RESEARCH REVIEW COMMITTEE (RRC)

[Please tick (✓) the appropriate box]

1. Has the proposal been reviewed, discussed and cleared at the Division level ?

   Yes ☑

   No ☐

   If 'No', please clarify the reasons: _____

2. Has the proposal been peer-reviewed externally ?

   Yes ☑

   No ☐

   If the answer is 'NO', please explain the reasons: _____

3. Has the proposal scope to address gender issues ?

   Yes ☐

   No ☑

   If the answer is 'YES', have these been adequately incorporated in the proposal. Please indicate: _____

4. Has a funding source been identified ?

   Yes ☑

   No ☐

   If the answer is 'YES', please indicate the name of the donor: _____

   Thrasher Research Fund

5. Whether the proposal is a collaborative one ?

Yes ☐

No ☑

If the answer is 'YES', the type of collaboration, name and address of the institution and name of the collaborating investigator be indicated:

_____

_____

_____

6. Has the budget been cleared by Finance Division ?

Yes ☑

No ☐

If the answer is 'NO', reasons thereof be indicated: _____

_____

_____

7. Does the study involve any procedure employing hazardous materials, or equipments ?

Yes ☐

No ☑

If 'YES', fill the necessary form.

27.5.96
Date

Rashidul Haque
Signature of the
Principal Investigator

APPLICATION FOR PROJECT GRANT

1.	INVESTIGATORS	:	Dr. Rashidul Haque Mr. M. A. Wahed. Dr. M. J. Albert ICDDR.B
2.	CONSULTANT	:	Prof. J. O . Alvarez Chairman Department of International Health and Nutrition Sciences University of Alabama at Birmingham
3.	TITLE OF PROJECT	:	Field trial of beta carotene and anti-helminthic therapy to improve micronutrient nutriture among preschoolers in Bangladesh.
4.	STARTING DATE	:	August 1, 1996
5.	DATE OF COMPLETION	:	One year and six months after starting
6.	TOTAL BUDGET REQUESTED	:	US $ 55,814
7.	FUNDING SOURCE	:	Thrasher Research Fund
8.	HEAD OF PROGRAMME	:	Director, Laboratory Sciences Division

ABSTRACT SUMMARY

Both intestinal parasitic infestations and micronutrient deficiency (vitamin A) are very common in children in the developing countries. Environmental interventions to interrupt of intestinal parasitic diseases constitute the ultimate solution to the problem of parasitic infestation of these children but is is the long-term objective and requires a lot of resources. On the other hand dietary interventions are the long-term objective to reduce micronutrient deficiency in the community. The new knowledge of the effects of parasitic infestation on the health (including vitamin A deficiency) of children and the recent development of effective and safe therapy has made the strategy of mass treatment of children attractive.

This proposal describes a field trial comparing the effects of daily supplementation with ß-carotene and/or anti-helminthic therapy (albendazole) on the vitamin A nutriture of preschoolers. The goal of the trial is to establish whether one intervention is more effective than the other, or whether a combination of these interventions is required to improve the vitamin A and overall nutritional status of preschool children.

The results that will be obtained from this study will provide useful information to health and nutritional programme planners with respect to the potential benefits from improved dietary intake of vitamin A (ß-carotene) and/or deworming. Considering the magnitude of the problem, the project finding will be of great importance in designing strategies for action oriented intervention in Bangladesh as well as in other developing countries where both vitamin a deficiency and intestinal parasitic infections are common.

10. OBJECTIVES

The major objective of the study is to examine the separate and combined impacts of low-dose β-carotene supplementation and anti-helminthic therapy on the vitamin A status and physical growth of preschool children in Bangladesh.

11. BACKGROUND

Importance of Micronutrient to Child Survival

The importance of micronutrients for ensuring the health, development and survival of infants and children is increasingly being recognized. Randomized controlled trials in several countries have provided evidence that deficiencies of vitamin A have serious and life threatening consequences [1,2,3]. It is now recognized that removing vitamin A deficiency as a public health problem can improve growth, and child morbidity and mortality [1,2,3,4,5].

Improving the health and wellbeing of children is a priority of the public health policy of many countries. Thus, combating vitamin A deficiency fits well within this policy. The following sections describe in more detail the epidemiology and public health importance of vitamin A deficiency in developing countries, and the importance of considering the role that parasitic infestation may play in diminishing the impact of dietary interventions.

Vitamin A

Vitamin A has been shown to be an important micronutrient for sight, growth, reproduction and immune function. Recent reports of the effect of vitamin A supplementation on childhood mortality

have suggested that improving vitamin A nutriture should also be considered as a child survival strategy. Many children in the developing world start life in a precarious nutritional state and because of poverty-related factors such as poor hygiene, poor diets and high morbidity loads, these children remain at risk for general and micronutrient-specific malnutrition.

Multiple factors affect vitamin A status in the individual; two of the most important are availability of dietary sources and adequacy of intake and absorption. Populations at risk of developing xerophthalmia, clinical vitamin A deficiency, appear to receive most, if not all, of their vitamin A from carotene-rich fruits and vegetables. Such foods tend to be seasonal, necessitating intake at many times the normal daily requirement in order to ensure that there are adequate liver stores which are needed to cover those periods when availability and consumption are low. Because their diets lack sufficient vitamin A and/or there is impaired absorption, one million preschool age children worldwide develop severe eye disease (xerophthalmia) each year. Of those children who develop corneal disease, as many as 85% may die [13].

Surveys have been conducted in many countries that provide information on the extent of the problem of vitamin A deficiency. Xerophthalmia is often associated with rice based diets in Asia. Investigators from several Asian countries have reported results of surveys that indicate that vitamin A deficiency is a problem in all parts of their countries. These countries include Indonesia, the Philippines, India, Bangladesh, Nepal and Thailand [6,7,8,9,10,11]. The body of evidence suggests that, beyond those with frank xerophthalmia, children with a 'sub-clinical state' of vitamin A nutriture may also benefit from vitamin A interventions.

Dietary Interventions

Various strategies have been implemented to eradicate vitamin A and iron deficiencies world wide, including tablet or capsule supplementation, food fortification, and education to promote consumption of micronutrient rich foods. Most of the public health efforts have focused on the first two strategies. The third means of intervention is neither quick, nor simple, but represents a sustainable public health measure for making lasting improvements on the vitamin A nutriture of all members of society. Foods that are promoted are those that would help meet nutritional requirements and that are both readily available and culturally acceptable. For vitamin A, these foods typically contain vitamin A in the form of *a*-carotene, yellow and orange fruits and vegetables and dark green leafy vegetables. Target groups include pregnant and lactating women, preschool children, and sick children. A recent study has shown that pure beta carotene supplementaion are much more effectve than diet (i.e., green leafy vegetables, stir fried) in improving the viatmin A status of pregnant women [12].

Parasitic Infection in Micronutrient Malnutrition

The effectiveness of such programs to improve vitamin A nutriture is threatened, however, in areas where intestinal parasites are common. Intestinal parasites, particularly round worms (Nematoda), impair absorption of many nutrients, including vitamin A, and thus may render improvements in dietary intakes of vitamin A ineffective. Further, it may be true that populations suffer from such deficiencies solely because of decreased absorption of a micronutrient adequate diet due to intestinal parasites; this problem has not been adequately addressed in the literature.

Intestinal parasites are a common but often 'silent' problem in the developing world. The prevalence of ascariasis in the tropical world is estimated to exceed 50% with children between the ages of 3 and 8 having the highest point prevalence. In regions experiencing a distinct wet and dry season, ascariasis is transmitted more readily during and just after the rains. Reinfection after successful treatment is very common and children experience more symptoms than adults [13]. In Bangladesh various studies suggest that 90 - 95% of children aged 6 -10 years had at least one parasite. The prevalence of *Ascaris lumbricoides*, *Trichuris trichiura* and *Ankylostoma duodenalis* were90-95%, 50-60% and 6-17% respectively in rural school aged children [14]. Intensity of A. *lumbricoides* infection is at its peak in children aged between 4-15 years and rises rapidly in the 2-4 years age group [15].

The impact of intestinal parasites on the nutritional status of children has been shown to be significant. There are several reports suggesting that children with various intestinal parasites, particularly Ascaris sp., have an impaired absorption of protein and fat [16,17,18,19]. Some researchers have found a relationship between indicators of micronutrient deficiency and infestation with *Ascaris lumbricoides* . Hematocrit, hemoglobin, plasma vitamin A and plasma carotenoid were significantly lower among infected children aged 3-6 years in Panama [20]. Children in India who were infested with *Ascaris lumbricoides* had significantly impaired absorption of retinol compared to normal controls [21] though a subsequent study by one of the authors failed to show that deworming was useful in raising serum retinol[22]. In 13 out of 14 adult study subjects with ascariasis, vitamin A absorption improved significantly after deworming; the authors suggested deworming as one means of improving vitamin A status [23]. Lastly, school aged Kenyan children grew better as assessed by several anthropometric measures when treated with anti-helminthic [24]. Importantly, this positive effect of deworming on nutritional status was observed even though the objective of the anti-

helminthic treatment was not that treated [illegible] would be "parasite free" during the course of the study.

12.RATIONALE

Vitamin A deficiency is an important public health problem and eradication of this deficiency will do much to promote the health and well-being of those afflicted. Although not simple and straight forward, dietary interventions are the only means of introducing long term dietary changes in communities that will remove such deficiencies as public health problems and prevent their reoccurrence. Parasitic infestation, particularly of helminths, may prevent improvements in dietary intake of vitamin A from showing the expected impact. Thus, program planners may need to consider deworming as a necessary component of dietary intervention strategies. Deworming itself, however might be seen as a logistically simple and frequently needed; but relatively inexpensive intervention for improving vitamin A nutriture. Marginal benefits of deworming, such as improved macronutrient absorption and improved growth and development must also be considered. Before embarking on one set of strategies for improving vitamin A nutriture, it would seem reasonable to determine the relative impacts on micronutrient status of deworming and improving dietary intake among children who have a marginally adequate diet with respect to vitamin A. At least one report from a study in Indonesia has shown that a such a combined dietary and deworming intervention can improve vitamin A status [25].

This proposal describes a field trial comparing the effects of daily supplementation with β-carotene and/or one time anti-helminthic therapy (albendazole) on the vitamin A nutriture of preschoolers. The goal of the trial is to establish whether one intervention is more effective than the other , or whether a combination of these interventions is required to improve the vitamin A and overall nutritional status of preschool children.

13. STUDY DESIGNS AND METHODS

Hypotheses

a) Deworming with albendazole will a) increase growth (relative to controls) as measured by a difference in the change in weight, height and MUAC over the interval of the trial and b) increase serum retinol and serum ß-carotene levels.

b) A dose of ß-carotene, equivalent to one RDA for vitamin A, on a daily basis will improve serum retinol and ß-carotene levels.

c) Deworming and daily ß-carotene in combination will improve growth, serum retinol and ß-carotene levels more than either intervention individually.

A. STUDY DESIGN

The study will be a double-masked, placebo controlled, clinical trail with four treatment groups as outlined in the table in page 10. Children will be individually randomized into one of four groups during a baseline assessment of health, nutrition, demographic and socioeconomic factors. The treatment groups will be Group 1, albendazole and daily ß-carotene supplementation, Group 2, albendazole and daily placebo, Group 3, placebo and daily ß-carotene supplementation, and Group 4, placebo and daily placebo.

The focus of this study will be on helminths namely *Ascaris lumbricoides* and *Trichiuris trichiura* beacuse these two helminths are very common in Bangladeshi children. Faecal egg counts of *A. lumbricoides* and *T. trichiura* will be used as an indicator of intensity of infection (worm burden) and a mean of three egg counts over a period of a few days will be used for this purpose. Faecal egg counts of worms will be expressed as egg per

and heavily infested with those helminths. Children with moderate to heavy infestations will be selected into the study and will be allocated randomly into one of the four treatment groups.

The albendazole will be a one-time dose of 400 mg; the placebo will be tablets of similar shape and color. Subjects in the placebo group will receive albendazole at the end of the trial. The ß-carotene intervention will be a capsule containing ß-carotene, approximately one RDA for preschool children in retinol equivalents. The placebo will be a similar capsule given daily.

Outcome measures will include serum levels of retinol and ß-carotene, and quantitative assessment of stool for intestinal parasites. Additionally, anthropometry, including length or height, and weight, will be performed at baseline and at six, and twelve months to assess growth. All children will receive albendazole and vitamin A at the close of the study, after twelve months of surveillance.

Subjects will be preschool children aged 2-4 years because they are at the greatest risk for both nutritional deficiencies and for intestinal parasitic infestations. Moreover, the age range represents one of the fastest growth periods making anthropometric monitoring more likely to yield important differences among the four groups.

	Anti-helminthic Treatment	ß-carotene Supplementation
Group 1	Albendazole	Daily ß-carotene tabs
Group 2	Albendazole	Placebo
Group 3	Placebo	Daily ß-carotene tabs
Group 4	Placebo	Placebo

Sample Size

The following formula is used for sample size estimation:

$$n = \frac{(\alpha + \beta)^2 (\sigma_1^2 + \sigma_2^2)}{(\mu_1 - \mu_2)^2}$$

where α = 0.95 (two sided), 1-ß = 0.90, and taken from Indonesian trial of Jalal et al.[25], σ_1 =1.28, σ_2 = 1.34, μ_1 = 22.58, and μ_2 = 24.51. This gives a sample size of 10 per group or 40 total. If the total sample size is increased by 25% for design effect and one assumes that there may be 10% attrition, the total sample size estimation becomes 14 per group or 56 total. If the sample size were to driven by the hypothesis related to growth and a difference of 30% is necessary for public health importance, there would be a large number of subjects required. Using the same α and ß, and assuming a coefficient of variation for growth of 40% in this age group, then a sample size of 37 per group is

required. Similar inflations for design effect and attrition result in a total sample size of 204, or 51 per group. Thus, if we enroll 204 subjects, we will have the power to test all hypotheses.

Inclusion criteria

Subjects will be preschool children (aged 2-4 years) because they are at the greatest risk for both nutritional deficiencies and for intestinal parasitic infestations. Moreover, this age range represents one of the fastest growth periods making anthropometric monitoring more likely to yield important differences among the four groups. Only children with demonstrated moderate to heavy *A. lumbricoides* and *T. trichura* infections will be included in the study. Parental consent will be obtained at baseline.

Exclusion criteria

Subjects with clinically apparent and significant vitamin A deficiency and/or severe malnutrition will be referred to the local health care unit for vitamin A supplements and excluded from the trail. Additionally, subjects who are currently being treated with either vitamin A or anti-helminthic medication will be excluded.

The study population

The study population will consist of children of both sex aged 2-4 years, living in Mirpur, a suburb of Dhaka, Bangladesh where prevalence of *A. lumbricoides* and *T. trichiura* is very high. The study area is well connected with the ICDDR,B with a population of fifty thousand or more.

B. METHODS

History and Physical examination

A list of all children with the above age range will be prepared from the area. After informed consent is taken from the head of the households, the mothers of these children will be interviewed at their houses by trained Health Assistants, using a standardized and pretested questionaree regarding the children's past illness. Detailed physical examination will be conducted by a medical officer after the interview.

Anthropometry

History and physical examination will be followed by Anthropometric measurements:

- Stature (accurate to the nearest 0.1cm), using a locally constructed stadiometer.
- Weight (accurate to the nearest 0.1 kg), using a high quality scale.
- Mid-upper-arm circumference (accurate to the nearest 0.1cm), using a flexible and non-stretchable tape.

Methods of taking weight and height and their standardization will be followed as described by the WHO [26]. Anthropometry will be performed by well trained and experienced Health Assistants in the presence of a physician and an average of three measurements will be considered as the observed value.

Screening for helminthic infections

Stool samples will be collected in a wide-mouthed plastic bottle for microscopical examination. Stool samples will be examined by microscopy using a wet smear and by a modification of the formol ether sedimentation technique [27]. Briefly, about 1-2 g of the faeces will be fixed in a weighed bottle containing 10 ml of 10% V/V formalin in saline and then weighed again to an accuracy of 0.1 g in order to determine the weight of faeces in the formol saline. Using a swab stick faecal sample will be broken into pieces and then will be mixed throughly on a vortex mixer after that sample will be filtered according to established method in our laboratory. After completing necessary treatment of the stool samples, finally stool samples will be treated with ether and then stool samples will be centrifuged at 3,000 rpm for 3-5 min. After decanting carefully the debris, using a clean Pasteur pippette a few drops of normal saline will be added to the sediment obtained after centrifugation and the total numbers of drops (TD) remaining in the tube will be counted. One or two drops from the tube (sample drop) will be taken onto a clean glass slide for microscopic examination. The whole slide will be examined carefully at a magnification of × 100 and the number of helminth eggs (HE) will be counted for each species (*A. lumbricoides* and *T. trichiura*) with the help of a hand tally counting chamber. Helminths eggs will be expressed in eggs per gram faeces, and the presence of only infertile Ascaris eggs will also be noted. The calculation of helminth eggs per gram of faeces will be done using the following formula:

$$\text{Eggs per gram (epg)} = \frac{TD/SD \times HE}{\text{Wt. of stool}}$$

Where, TD = Total drops in the tube
SD = Sample drops examined
HE = Number of helminth eggs

Biochemical methods

Blood (1ml) will be collected by venipuncture. Serum will be separated and stored at $-70^{O}C$ until analysis. Both serum retinol and ß-carotene will be measured using HPLC following the methods by DeRuytu et al [28] and Handleman et al[29].

Data analysis

Analysis of the data obtained in this study will require the use of several statistical methods and procedures. Initially, univariate analyses will be performed on a variety of study variables to determine the comparability of the four treatment groups. Recalling that we will utilize a factorial design (i.e. AB, Ab, aB, ab), where uppercase letters represent a treatment and lower case letters represnt the placebo), it will be important to show that the randomization process resulted in four groups which are similar with respect to key baseline variables such as gender, age, anthropometric status, vitamin A status and percent with worm infestation.

A second level of analyses would be an application of analysis of varience (ANOVA) to determine, for instance the mean change in serum retinol levels from baseline to six months by group and whether there is a difference between groups. Fisher's probability of least significant difference (LSD) and/ or the Scheffe's f-test could then be applied to determine where (between which groups) significant differences occur.The results from these analyses would determine whether and the degree to which either ß-carotene supplementation or deworming improved outcome measures, i.e growth and vitamin A status.

A third series of analyses would employ multiple regression analyses where treatment A would be assigned the value 1 and

placebo would be 0, treatment B would be 1 and placebo 0. Regressions could be run with two variaables (treatment A and B) but perhaps more importantly for three variables (treatment A and B and an interaction variable A*B).

Lastly, additional factors could be included in multiple regressions as a way of controlling for confounding variables and addressing important interactions. Since our aim is to provide useful information to health and nutrition program planners with respect to the potential benefits from improved dietry intake of vitamin A (ß-carotene) and/or deworming, this last series of analyses will make use of the spectrum of our study population. Controlling for several baseline variables will result in inference which may help direct such an intervention(s) to target groups who might benefit the most.

14. STUDY SCHEDULE

Once informed consent is given, baseline sociodemographic and anthropometric data will be gathered. Stool examination for three time for determination of intensity of helminthic infection will be carried out and only moderate to heavily infected children will be selected. Additionally, serum for retinol, ß-carotene will be measured. Subjects will be given a unique study number which will also determine the subject's treatment allocation (albendazole or placebo, ß-carotene or placebo). At the close of the baseline evaluation, two groups of children will receive a dose of albendazole thrice, that is, at the begining of the study , at 4 months and at 8 months and the other two groups placebo. The ß-carotene or placebo treatments will be daily doses delivered to the home in packets of seven on a weekly basis. ß-carotene or placebo treatments will begin within one week of the albendazole/placebo treatment.

The field workers will make weekly visits to each home to monitor the progress of the study. At this time the field workers will deliver packets of a-carotene or placebo.

The interval of follow up will be six months. At six and tweleve months of observation, the study subjects will be weighed, measured and stools will be collected for parasitological examination. Venous blood will be collected for serum retinol and beta carotene, at baseline, 6 months and at the end of the study. As a subject graduates from the study s/he will receive doses of vitamin A and albendazole.

Flow chart of the workplan :

Months 0___2___3___4___5___6___7___8___9___10___11___12___13___14___15___16___17___18___

Screening &

selection of_____

children

Recruitment

of children _____

Treatment &

Follow up _____

Laboratory

procedures _____

Data analysis _____

DETAILED BUDGET (for 18 months)

I. Local Salary		Amount in US Dollar
I.1 Dr. Rashidul Haque	40% NOB	6,866
I.2 Mr. M A Wahed	10% NOC	2,381
I.3 M.J Albert	2% P5	2,133
I.4 Lab. technician	100% GS-III	4,533
I.5.Lab attendant	100% GS-I	2,000
I.5 CHW (3)	100%	6,000
Sub-Total		23,913
II. Supplies, materials and tests:		
II.1 Office supplies		1,200
II.2 Chemical, reagents and supplies for measurement of serum retinol and beta carotene		7,500
II.3 Chemical ,reagents and supplies for parasitological examination		3,500
II.4 Albendazole and placebo		300
II.5 ß-carotene and placebo		5,000
Sub-Total		17,000
III. Other contractual Services:		
III.1 Repair Maintainance		500
III.2 Telephone/Fax/Postal		700
III.3 Photocopying and mimeography		1,200
III.4 Data entry		2,000
Sub-Total		4,400
IV. Travel		
IV.1. Local Travel		1,350
IV.2. International Travel		2,500
Sub-Total		3,850
V. Patient care costs Remuneration to the study subjects and other care		3,000
Total Operating Cost		52,163
Added Overhead (7% of direct cost)		3,650
Total costs		55,814

01/06/96

16. REFERENCES

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International Centre for Diarrhoeal Disease Research, Bangladesh

CONSENT FORM

Project: Field trial of beta carotene and anti-helminthic therapy to improve micronutrient nuriture among preschoolers in Bangladesh.

Many children in Bangladesh are infected with the intestinal worm Ascaris, called "kechucrimi" in Bangla and are also suffering from vitamin A deficiency. Vitamin A deficiency is an important public health problem in Bangladesh and eradication of this deficiency will do much to promote the health and well-being of those afflicted. We are doing a study to examine the separate and combined impacts of low-dose beta carotene supplementation and anti-helminthic therapy on the vitamin A status and physical growth of preschool children in Bangladesh. This could form the basis of a new policy to combat this problem. For this work we need to collect stool from a number of children infected with worms. Please will you allow us to collect stool samples from your children.

If we find that your children is infected with moderate to heavy worms then your children will be selected for the study. If you allow your children to participate in the study then the following interventions and procedures will be carried out during the 1 year period of follow-up.

1. We will take weight and height of your children, and will take 2ml of blood sample at the beginning of the study.

2. Your children will be given treatment according to the study design (Albendazole or placebo and daily beta carotene or placebo)

3. At six months stool sample and 2ml of blood will be collected for parasitological examination and for measurement of serum vitamin A. At this time weight and height will also be taken.

4. Treatment will be continuing for one year according to study design.

5. At the end of the study after 1 year of surveillance blood and stool sample will be collected again.

6. As a children graduates from the study s/he will receive doses of vitamin A and anti-helminthic therapy.

(To be read out to parents whose children are taking part in the study)

There is no obligation to take part in this study. Also, if you want to take out your children from the study you can do that any time during the study.

If you agree that your children can participate in this study, then please sign below or mark the paper with your finger print.

Thank you.

Signature or fingerprint
of child's parent:

Signature of Investigator:

Date:

Title: "Field trial of B-carotene and anti-helminthic therapy to improve micronutrient nutrition among preschoolers in Bangladesh"

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

Position:

Institution:

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: .....

Reviewer I

Critique:

The study has 2 clearly different objectives, as stated by the authors, to assess the effect (separately and combined) of low-dose daily β-carotene supplementation and anti-helminthic therapy on (a) vitamin A status, and (b) physical growth.

The first objective is straight forward and should be studied. However, this effect can be assessed in a period much shorter than one year, i.e., 3-6 months.

The second objective of the study can generate several criticisms: (i) is the age of 2-4 yrs. the most appropriate one to study improvement of linear growth since growth rate is much greater from birth to 2 yrs. Moreover, many studies have documented that most of the stunting in children occurs before age 2 yrs and there is little catch-up growth after that age (see J. nutr. April 1995 supplement, vol. 125 number 4S). (ii) is it ethical to extend a study to 1 year and not treat children (see placebo-placebo group) infested with parasites, particularly those with a moderate to heavy parasite load while just observing their growth?

In summary, the project as submitted justifies a short study to evaluate the impact of β-carotene and parasite treatment on vitamin A status (6 months). Extending the project to 12 months to assess the impact on growth is unwarranted unless a stronger justification is presented and the design of the study modified accordingly (for example, the age of the cohort). The budget should be reduced if the duration and aims of the study are shortened.

The methods proposed are quite adequate. Aside from the main criticism regarding the second objective of the study, the project is well written, the design of the study is adequate and overall, the proposal is quite good.

Reviewer II

Comments on "Field Trial of B-carotene and anti-helminthic therapy to improve micronutrient nutriture among preschoolers in Bangladesh"

This is a well written, understandable proposal. The design, rational and basis of the proposal seems straightforward.

There are some questions, however. There seems to be an emphasis both in the literature and here on ascaris as the major cause of Vit A deficiency but in actuality strongyloidiasis(not studied) or giardiasis(shown in other studies) can effect Vit A absorption. It might be worthwhile to consider other causes and officially consider them in the analysis. Albendazole has effects on many helminths and protozoa(although at longer duration and increased dose); previous authors' have concluded most of the effects of treatment are due to elimination of ascaris but no one has ruled out effects on other parasites which may be treated effectively or partially with albendazole. Nevertheless, whatever the culprit the design of the trial should allow a rather even distribution of pathogens.

Presumably, the type, degree, and age specific prevalences of gastrointestinal parasites are well defined in the study population; it isn't mentioned anywhere in the proposal.

It is unclear why the authors' choose 2 treatments instead of 4 for instance. If reinfection rates are high then effects might be lost or harder to show. I would consider using every 3 or 4 month treatments. The authors' need to show a positive effect and can work backward to show the minimal necessary treatments if need be.

This is an good fundable project.

Response to reviewers comments:

Reviewer I:

The reviewer has commented on the importance of conducting the study. He has mentioned very clearly that the study has 2 clearly different objectives, to assess the effect (separately and combined) of low-dose daily beta carotene supplementation and anti-helminthic therapy on (a) vitamin A status, and (b) physical growth.

The reviewer believes that the first objective is straight forward and should be studied. We are agreed partially with the reviewer that this effect can be assessed after 6 months but, it would be very important if it could be assessed after twelve months since, intestinal helminthic infections are chronic to see an effect to improve vitamin A status it might take longer time.

The criticisms of the reviewer on the second objective is not consistent with the objective of the project. The study subjects will be children aged 2-4 years because they are at the greatest risk for both nutritional deficiencies and for intestinal helminthic infestations. Although it would have been nice to choose a narrower age range for this study, say 1-2 years to obtain more precise anthropometric data, it would be hard to get sufficient children with moderate to heavy infestations in this age group. The age range 2-4 years is considered appropriate for this study.

The evaluation of growth as an outcome would be strengthened if the study period were longer. A 12 month study period would allow more time for children to benefit in terms of growth, and would also capture the effect of the intervention across all seasons.

Reviewer II:

The reviewer is agreed with the design, rational and basis of the proposal. However, he has mentioned about the role of other parasites in causing vitamin A deficiency. The primary objective of this proposal is not to look at the role of different parasites in causing vitamin A deficiency but, to look at the effect (separately and combined) of low-dose daily beta carotene supplementation and anti-helminthic therapy on (a) vitamin A status, (b) physical growth. We have clearly mentioned inclusion criteria for recruitment of the children. Since, all other helminths and protozoa present in the stool samples of these children will be recorded during microscopic examination, it would not be a problem to consider them if necessary in the analysis.

We have now mentioned in the background and references information about the prevalence of gastrointestinal parasites.

Regarding the anti-helminthic treatment we have taken into consideration of the reviewer comments and we will treat the children at the baseline and every four months.