ETHICAL REVIEW COMMITTEE, ICDDR,B.

Date 22 April, 19

Principal Investigator JILEMENS

Trainee Investigator (if any) JJ

Application No. 84-019(P)

Supporting Agency (if Non-ICDDR,B)

Title of Study Safety of killed K12 Elix Strain Prepared as Placebo for oral Cholera vaccine Trial

Project status: PILOT
( ) 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 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 Committee:
   ___ Umbrella proposal - Initially submit overview (all other requirements wil be submitted with individual studies
   ✓ Protocol (Required)
   ✓ Abstract Summary (Required)
   ✓ Statement given or read to subjects nature of study, risks, types of que ions to be asked, and right to refus to participate or withdraw (Required
   ✓ Informed consent form for subjects
   ___ Informed consent form for parent or guardian
   ___ Procedure for maintaining confidenti ity
   ✓ Questionnaire or interview schedule

* If the final instrument is not complete prior to review, the following informati should be included in the abstract summa
   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 sensiti areas.
   3. An indication as to when the questio naire 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.

Principal Investigator — April 22, 1984

30 APR 1984

SECTION I - RESEARCH PROTOCOL (PILOT)

1. Title: Safety of killed K12 E. coli strains as placebo for oral Cholera Vaccine Trial.

2. Principal Investigator: Dr. John D. Clemens

   Co-Investigator: Mr. M.R. Khan

3. Starting Date: May 15 1984

4. Completion Date: July, 15 1984

5. Total Direct Cost: US$2907.98

6. This protocol has been approved by the Disease Transmission Working Group (DTWG) Working Group.

   Signature of the Scientific Program Head: K.M.S. Azeez

   Date: 22/4/84

7. Abstract Summary:

In order to conduct the proposed Field Trial of Oral B-subunit/whole-cell (BS/WC) and Whole-cell (WC) vaccines in a double-blinded fashion it will be necessary to develop an adequate placebo which closely resembles the two vaccines in taste appearance, and smell. It has been determined by the developers of the vaccines that only a killed bacterial preparation can fulfill these characteristics. An ideal candidate bacterium is the K12 strain E. coli, which is known to be non-pathogenic and is well-characterized A K12 strain which is devoid of pathogenic factors (ST, LT production and plasmids) and which does not evoke antibodies which cross-react with V. cholerae will, after safety-testing in Swedish volunteers, be safety-tested in Bangladeshi volunteers 150 children (aged 2 14 years and female adults over 14 years) will be randomly allocated to receive either the placebo or distilled water, mixed with a bicarbonate citric acid solution to be used in the trial. Each volunteeer will be

followed an 3 successive days after receipt of the agents for the occurrence of side-effects. Follow-up will be blinded to the identity of the received agent.

Reviewers:

(a) Research Involving Human Subjects: _____

(b) Research Review Committee: _____

(c) Director: _____

(d) Controller/Administrator: _____

SECTION II - RESEARCH PLAN

A. INTRODUCTION

At a recent meeting of ICDDR,B, WHO, Institute Merieux, The Swedish Bacteriology Laboratory, and Drs. David A. Sack and Jan Holmgren, held in Lyon, France, March 19-20, it was unanimously agreed that a killed bacterial placebo would be necessary for the forthcoming field trial of Oral B-subunit/whole-cell (BS/WC) and whole-cell(WC) vaccines.

The placebo planned is a heat killed E. coli strain K12. This strain was chosen because it is a strain well characterized in the laboratory. It is used extensively to receive virulence factors located on plasmids because it has none of its own. For example, it has been used to receive a heat-labile toxin plasmid, heat-stable toxin plasmid, or receive a plasmid responsible for antibiotic resistance(1). Before receiving the respective plasmids, it had neither LT, CFA nor antibiotic resistance R factor. We propose to use the parent K12 strain e.g. the strain without any virulence factors.

Furthermore, we will heat-killed the bacteria before using it as a placebo so that the participants will receive only a sterile preparation. Sterility will be documented at Institute Merieux and at the Swedish Bacteriology Laboratory.

Killing the bacteria by heating will, of course, insure sterility. Also it will destroy or alter the bacterial portions to minimize the already negligible chance of any cross-reacting proteins between the E. coli

placebo and the cholera vaccine. To document the absence of any such virulence factors, the placebo will be tested in the following ways at the Institute Merieux and at SBL. It should produce no fluid accumulation in the rabbit loop model, the infant mouse model, nor should it stimulate Y1 adrenal cells. No ST toxin should be detectable with gene probe assay, and no plasmid should be demonstrable with plasmid electrophoresis. When used to immunize rabbits, it will be shown to stimulate no anti-cholera antibodies.

In addition, the K12 placebo will be tested in Swedish volunteers using the same doses as anticipated in the field trial before being used in Bangladesh. In this study it will be tested for safety in a group of Bangladeshi volunteers prior to its use in the pre-trial.

We anticipate that the K12 placebo will have no toxicity for several reasons. First, being sterile, the bacteria cannot multiply nor colonize the human intestine. Secondly, killed E. coli are frequently consumed by humans without ill-effect. One of the reasons for cooking food or boiling water is to kill the coliform bacteria before consuming it and thereby eliminate the possibility of symptoms due to bacterial infection. Thirdly, K12 lacks all of the known virulence factors needed to cause enteric illness. Fourthly, live E. coli which lack these virulence factors (including both the K12 and the H.S. strains) have been given to volunteers without any side effects in doses up to 10^{10} live bacteria (2,3,4).

SPECIFIC AIMS

The specific aim of this study is to confirm the safety of a K12 *E. coli* solution for use in the pre-test for the field trial of BS/WC and WC oral Cholera Vaccines.

METHODS

1. Antigen:

A K12 *E. coli* solution with identical turbidity (approximately 10^{11} cells/total volume) to the BS/WC and WC liquid vaccines (which themselves are identical in appearance) will be stored in the 8 ml doses anticipated for the field trial. Before use in Bangladesh, the strain will be ascertained to be free of LT toxin, ST toxin and plasmids, using the following standard assays (Dr. Jan Holmgren's laboratory): the rabbit loop model, the infant mouse model, the Y1 adrenal cell assay, plasmid electrophoresis and a gene probe assay (for ST). This laboratory will also ascertain that the K12 strain does not raise antibodies in rabbits which cross react with *V. cholerae*. Identical doses of placebo will also be fed to Swedish volunteers to ensure freedom from side-effects before use in this study. Sterility will be ensured by culturing the K12 *E. coli* after it has been heat-killed.

2. Patients:

Eligibility for the trial will include: (a) age 2-14 years, or > 14 years and female (same age-gender eligibility as that for the trial), (b) absence of the following symptoms on the day of administration: diarrhoea, fever, vomiting, abdominal pain exanthanatous skin rash; (c) absence of pregnancy and (d) willingness to cooperate.

(Informed Consent Form is in Appendix I). Subjects will be recruited from villages V3 and V4 in Matlab.

Maneuvers:

On a random basis, subjects will receive either 8 cc of distilled water or 8 cc of the K12 placebo, mixed with bicarbonate citric acid in the following dose: (a) for children 2-10 years, 1.9 grams sodium bicarbonate and 7 grams citric acid in 75 ml water; (b) for persons > 10 years, 3.8 grams sodium bicarbonate and 1.4 grams citric acid in 150 ml water. Subjects will not be told which agent they are receiving their doses of acid-butter have been well tolerated in a past study of oral BS/WC vaccine by the Principal Investigator.

Sample size.

75 persons will be assigned to each of the two groups. This size will ensure detection of moderately frequent ($\geq$ 5% occurence) side-effects, with adequate ($\geq$8) statistical power.

Follow-up:

During the 3 days following receipt of the agents, each subject will be queried about the following symptoms (according to a standard form used in past studies of oral cholera vaccines at ICDDR,B): abdomina pain, fever, vomiting, diarrhoea (>3 loose motions/24 hours), skin rash and syncope. Subjects will also be asked to report additional symptoms not specifically noted on the form. Follow-up will be performed by teams experienced in field studies of oral vaccines, and the menbers of this team will be unaware of the assigned agent. A physician will be available to treat any severe side-effects, which, of course, are not anticipated.

6. Statistical analysis:

The comparative occurence of side-effects in the two groups will be assessed by chi square and Fisher's exact tests where appropriate.

SIGNIFICANCE

An adequate placebo which is safe for consumption is essential to the conduct of the planned oral cholera vaccine field trial. This study will provide evidence of the placebo's safety before use in the pre-test for the field trial.

Subjects aged 2-14 years and females aged over 14 years will be included, since these are the subjects to be included in the planned field trial of oral cholera vaccine.

Risk of side-effects from taking the killed K12 E. coli should be negligible, since the organisms will have been proved non-pathogenic by exhaustive testing and will have been shown to have no side-effects in Swedish volunteers.

Risks (side-effects) of the proposed E. coli placebo will be assessed on a daily basis, and all serious side-effects will be treated by a physician.

All data will be kept confidential by (a) identifying subjects in the analysis by number, not by name and (b) keeping all data locked in a filing cabinet in the principal investigator's office.

Signed informed consent will be obtained from all subjects (or parents of minors), prior to participating in this study. Treatment will be available for significant side-effects.

No interview will be required.

An excellent placebo is required for the cholera vaccine trial. This study will confirm the safety of the proposed placebo. The benefit of an well-executed vaccine trial is the valid evaluation of a vaccine which may be very beneficial in Bangladesh; the risks in this study are negligible.

No records, organs, tissues, body fluids, feti, or aborti will be used.

References

1. Echeverria P, Murphy JR. Enterotoxigenic Escherichia coli carrying plasmids coding for antibiotic resistance and enterotoxin production. J. Infect. Dis. 142:273-278, 1980.

2. DuPont HL, Formal SB, Hernich RB, Snyder MJ, Libonati JP, Sheahan DG, LaBrece EH, Kalas JP. Pathogenesis of Escherichia coli diarrhoea. New Eng J Med 285:1-9, 1971.

3. Levine MM, Berquist EJ, Nalin DR, Waterman DH, Hornich RB, Young CR, Sotman S. Escherichia coli strains that cause diarrhoea but do not produce heat labile or heat stable enterotoxins and are non-invasive. Lancet 1:1119-1122, 1978.

4. Levine MM, Kaper JB, Lockman H, Black RE, Clements ML, Falkow S. Recombinant DNA risk assessment studies in humans: efficacy of poorly mobilizable plasmids in biologic containment. J Infect Dis, 1983 Oct; 148(4): 699-709.

SECTION III - BUDGET

A. DETAILED BUDGET

PERSONNEL SERVICES

Name	Position	% of effort	Annual Salary	Project Requirements Taka	Dollar
Dr. John Clemens	P. Investigator	-	-	-	-
Mr. M.R. Khan	Co-Investigator	-	-	-	-
To be named	H. Assistant (5)	33%	$ 2,951	-	973.83
To be named	C.H.W. (3)	20%	$ 658	-	131.60
To be named	Porter (2)	15%	$ 658	-	98.70
		Sub-total:		-	1204.13

SUPPLIES AND MATERIALS

Miscellaneous (aspirin, candy cups, scabies medicine etc.)	-	250.00
Sub-total:	-	250.00

EQUIPMENT - None

PATIENT HOSPITALIZATION - None

OUTPATIENT CARE - None

ICDDR,B TRANSPORT

Speedboat (60 hours x 350 Tk./hr.)	21,000.00	-
Dhaka-Matlab-Dhaka (6 trips @ Tk. 1,250/trip)	7,500.00	-
Sub-total:	28,500.00	-

TRAVEL OF PERSONS - None

TRANSPORTATION OF THINGS - None

RENT - None

PRINTING AND STATIONARY:

Forms, Stencils, Xerox	-	150.00
OTHER CONTRACTUAL SERVICES - Data Analysis	-	150.00

CONSTRUCTION AND RENOVATION - None

B. BUDGET SUMMARY

			TAKA	DOLLAR
1.	Personnel Services	-	-	1,204.13
2.	Supplies and Materials	-	-	250.00
3.	Equipment	-	-	-
4.	Patient Hospitalization	-	-	-
5.	Outpatient Care	-	-	-
6.	ICDDR,B Transport	-	28,500.00	-
7.	Travel of Persons	-		-
8.	Transporation of Things	-	-	-
9.	Rent	-	-	-
10.	Printing and Stationary	-	-	150.00
11.	Other Contractual Services	-		150.00
12.	Construction & Renovation	-	-	-
		Total:	28,500.00	1,754.13

Grand Total: US$ 2907.98

Conversion Rate US$ 1.00 = Tk. 24.07

APPENDIX I

Consent Form

The International Centre for Diarrhoeal Disease Research, Bangladesh, plans to test a new oral vaccine against cholera next January. In order to test the vaccine, we need to give another substance which is harmless, but which is not itself a vaccine.

In this study, you/your child will drink either this non-vaccine substance (which consists of killed bacteria) or another harmless substance. We will ask you about any side-effects that youmight have for 3 days after receiving either substance. No blood will be taken.

You/your child do not have to participate in the study. If you decide not to join, your care at ICDDR,B will not be affected. You may also withdraw from the study at any time.

Any side-effect developing during the 3 days after drinking one of the substances will be treated by ICDDR,B.

I agree to cooperate with the study on my join/my child's behalf.

Signature : _____

_____ LTI : _____

Signature of the staff

Date : _____

APPENDIX II

Reaction Form

Name of subject: _____ DSS Number: _____

DOB: _____ Gender: _____

Date of Vaccination: _____ Subject Interviewed: Day 1 Y N

Day 2 Y N

Day 3 Y N

Interviewer: _____

Symptoms (Y N)	Day 1	Day 2	Day 3	Describe onset, severity and therapy
Abdominal Pain:				
Fever:				
Vomiting:				
Diarrhoea:				
Watery				
Non-watery: No blood				
Non-watery: Blood				
Rash:				
Syncope:				
Others (Name):				

সম্মতি পত্র

আন্তর্জাতিক উদরাময় ব্যাধি গবেষণা কেন্দ্র, বাংলাদেশ, আগামী জানুয়ারী মাসে কলেরা-নিরোধী একটি নতুন খাওয়ার ভ্যাক্সিন মূল্যায়নের পরিকল্পনা করছে। ভেক্সিনের পরীক্ষার জন্যে আমাদেরকে ভেক্সিন-নয় এমন আর একটি দ্রব্য দিতে হচ্ছে, যা ক্ষতিকারকও নয়।

এই নিরীক্ষায় আপনি/আপনার শিশু ভেক্সিন-নয় এমন একটি দ্রব্য (যাতে রয়েছে মৃত জীবাণু) অথবা অপর একটি নির্দোষ দ্রব্য পান করবেন। এর যে কোনো একটি নেবার পর তিন দিন পর্যন্ত কোনো পার্শ্বপ্রতিক্রিয়া দেখা দেয় কি না সে বিষয়ে আমরা আপনাকে প্রশ্ন করবো। কোনো রক্ত নেয়া হবে না।

আপনাকে/আপনার শিশুকে এই নিরীক্ষায় অংশ নিতেই হবে এমন কোনো কথা নেই। আপনি অংশগ্রহণ না করলেও আই সি ডি ডি আর বি-তে আপনার স্বাভাবিক চিকিৎসার কোনো ত্রুটি হবে না। নিরীক্ষায় অংশ নিয়েও যে কোনো সময় আপনি সিদ্ধান্ত প্রত্যাহার করে নিতে পারেন।

দু'টি দ্রব্যের যে কোনো একটি পান করার পর তিন দিনের মধ্যে যে কোনো পার্শ্ব প্রতিক্রিয়া দেখা দিলে আই সি ডি ডি আর বি তার চিকিৎসা করবে।

আমি নিজে/আমার সন্তানের পক্ষে এই নিরীক্ষায় সহযোগিতা করতে সম্মত।

স্বাক্ষর:----------

কর্মচারীর স্বাক্ষর:---------- বাম বৃদ্ধাংগুলির ছাপ:----------

তারিখ----------