

26

Principal Investigator David A. Sade Trainee investigator (if any) \_\_\_\_\_

Application No 78-006 Supporting Agency (if Non-CRL) \_\_\_\_\_

Title of study Milk Antibodies to E. coli-toxin and colonization factor in Bangladeshi women. 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):

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 possibly 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
  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 Board for review.

We agree to obtain approval of the Review Board on Use of Human Volunteers for any changes involving the rights and welfare of subjects before making such change.

David A. Sade  
Principal Investigator

\_\_\_\_\_  
Trainee

Rec'd 24/1/78  
78-006

SECTION I - RESEARCH PROTOCOL

- 1) Title: Milk antibodies to E. coli toxin and colonization factor in Bangladeshi women.
- 2) Principle Investigator: David A. Sack, M.D.  
Collaborating Investigator: Dolores G. Evans, Ph.D., Houston, Texas.
- 3) Starting Date: February 1978.
- 4) Completion Date: December 1978.
- 5) Total Direct Cost: \$2,193
- 6) Abstract Summary:

Milk from 50 Bangladeshi lactating women will be assayed for antibodies to E. coli heat labile toxin and E. coli colonization factor. The levels of antibody will be compared to milk samples from similar groups of women in the United States, Costa Rica and Mexico. This comparison will give an indication of the level of exposure of the enteric antigens which are important in disease in the different population groups, and will also begin to document the role that these antibodies play in protecting the nursing baby.

- 7) Reviews:
  - a) Research Involving Human Subjects: \_\_\_\_\_
  - b) Research Committee: \_\_\_\_\_
  - c) Director: \_\_\_\_\_
  - d) B-RC: \_\_\_\_\_
  - e) Controller/Administrator: \_\_\_\_\_

## SECTION II - RESEARCH PLAN

### A. INTRODUCTION

1. Objective: The object of this protocol is the assessment of the level of exposure to important enteropathogenic antigens in Bangladesh, as indicated by levels of antibody in breast milk and the comparison of these levels with other population groups.
2. Background: Enterotoxigenic *E. coli* diarrhea continues to be a major cause of diarrhea in Bangladesh among all age groups. Several studies from the CRL have documented the importance of this disease as a cause of both severe cholera-like diarrhea as well as less severe "outpatient diarrhea." The pathogenesis of the disease involves first the ingestion of pathogenic organisms, followed by colonization of the small intestine, then by elaboration of enterotoxins which results in secretion of fluid from the intestine. **A specific antigen has been identified, at least for *E. coli* 078 strains, which promotes the colonization of the ETEC, and hence is an important pathogenic factor.** 078 ETEC are known to be among the more common ETEC in Bangladesh and in fact, this colonization factor (CF) was isolated from a strain from Dacca (10407). This colonization factor has been shown to be a pilus structure and to be coded for by a plasmid, separate from the plasmid which codes for heat labile toxin (LT). The specificity of the "10407-type" CF for 078 strains is not yet clear, i.e., it is not known if all human ETEC have a similar colonization factor or if the presently identified CF is specific for 078. The importance of the "10407-type" CF would grow in relation to potential vaccines if it were a general feature of all ETEC.

As opposed to CF, the role of toxin is much more clear. By definition, all ETEC produce toxin regardless of serotype, and of the two known toxins (heat stable and heat labile) LT is the one known to be antigenic. While ETEC diarrhea (and LT) is known to be common in lesser developed countries, the role of the CF in countries of the western hemisphere is less clear.

The study of milk is becoming important because of the close immunologic relationship of milk to the gut in reflecting local immunity and in potentially protecting nursing babies. Exposure of the gut to antigens can be assessed by measuring antibodies to these antigens in breast milk and a comparison of antibody levels in milk should allow for an assessment of enteric antigenic exposure in the geographic areas. We would expect that antibody levels to LT and CF would be low in the U.S.A. since ETEC disease is uncommon. In Bangladesh the antibody levels to LT and CF would be higher. In Mexico and Costa Rica antibody to LT should also be higher than U.S.A. since ETEC is common; however, antibody levels to CF may or may not be higher depending on the importance of the CF antigen in that area.

3. Rationale: We plan to measure the antibody levels to CF and LT in milk samples of Bengali lactating women and compare these antibody levels to similar samples collected in Mexico, Costa Rica and the U.S.A. in order to assess the level of exposure of these enteropathogenic antigens in the population groups.

B. SPECIFIC AIMS

To measure antibody levels to LT and CF in milk samples from Bangladeshi lactating women and compare them to levels in milk samples from groups of women in U.S.A., Mexico and Costa Rica.

C. METHODS OF PROCEDURE

Fifty Bangladeshi women who deliver a baby at Dacca Medical College Hospital will be recruited for the study. The women will answer a short verbal questionnaire (see questionnaire enclosure) with the help of a field worker. Breast milk will then be collected (5-10 ml) by the mother by manual expression.

The milk will immediately be aliquoted into three aliquots and then snap frozen in liquid nitrogen, to be transported back to CRL. The samples will then be lyophilized. The milk samples will then be assayed for anti LT by the  $Y_1$  adrenal cell assay in Dacca and one aliquot will be sent to Dr. Dolores Evans who will assay the samples for IgA anti LT by Elisa assay and for IgA anti CF by Elisa assay. She will also be receiving samples from Costa Rica, Mexico and U.S.A. to be assayed in the same assays. Total IgA will also be measured so that antibody will be expressed as antibody titer per IgA.

Fifty patients from each of the geographic areas will be used. Both the geometric mean titer and the prevalence of detectable antibody will be compared between the geographic areas.

D. SIGNIFICANCE

This study would add to our understanding of the epidemiology of enterotoxigenic E. coli and the role of "10407-type" colonization factor in the world wide distribution of the disease.

E. FACILITIES REQUIRED

1. Office space is already provided.
2. Lab space is already provided.
3. Hospital resources: none.
4. Animal resources: none.
5. Logistic support: transport to Dacca Medical College.

6. Equipment: none.

7. Other.

F. COLLABORATIVE ARRANGEMENTS

Professor Syeda Feroza Begum, Gynecology and Obstetrics Unit I has approved the collection of milk samples from her unit.

Dolores G. Evans, Ph.D. will be performing the colonization factor and LF ELISA assays in Houston, Texas.

SECTION III - BUDGET

A. DETAILED BUDGET

1. PERSONNEL SERVICES

| <u>Name</u>                  | <u>Position</u> | <u>Percent of effort<br/>or number of days</u> | <u>Annual<br/>Salary</u> | <u>Project Requirements</u> |                |
|------------------------------|-----------------|--|--------------------------|-----------------------------|----------------|
|                              |                 |  |                          | <u>TAKA</u>                 | <u>DOLLARS</u> |
| David Sack                   | Investigator    | 5%   | \$ 34,750                |                             | 1,737.50       |
| Mr. Neogi                    | Lab Technician  | 5%   | Tk.20,000                | 1000                        |                |
| Epidemiology Field Assistant |                 | 5%   | Tk.20,000                | 1000                        |                |
|                              |                 |  | Sub Total:               | 2000                        | 1,737.50       |

2. SUPPLIES AND MATERIALS

| <u>Items</u>                          | <u>Unit Cost</u> | <u>Amount Required</u> |            |         |
|---------------------------------------|------------------|------------------------|------------|---------|
| Collection vials                      |                  | 150                    |            |         |
| Liquid nitrogen                       |                  |                        |            |         |
| Time on lyophilizer<br>and centrifuge |                  |                        | 200        | 100     |
| Tissue culture plastics<br>and media  |                  |                        |            |         |
|                                       |                  |                        | Sub Total: | 200 100 |

3. EQUIPMENT

None

4. PATIENT HOSPITALIZATION

None

5. OUTPATIENT CARE

None

|     |  | <u>Project Requirements</u> |                |
|-----|--|-----------------------------|----------------|
|     |  | <u>TAKA</u>                 | <u>DOLLARS</u> |
| 6.  | <u>CRL TRANSPORT</u>                         |                             |                |
|     | Milage-Dacca: 100 miles                      | 340                         |                |
|     | Milage-Other: none                           |                             |                |
|     | Hours-Water Transport: none                  |                             |                |
|     |  | _____                       | _____          |
|     | Sub Total:                                   | 840                         |                |
| 7.  | <u>TRAVEL AND TRANSPORTATION OF PERSONS</u>  |                             |                |
|     | None   |                             |                |
| 8.  | <u>TRANSPORTATION OF THINGS</u>              |                             |                |
|     | None   |                             |                |
| 9.  | <u>RENT, COMMUNICATIONS &amp; UTILITIES</u>  |                             |                |
|     | Postage                                      |                             |                |
|     |  | _____                       | <u>20</u>      |
|     | Sub Total:                                   |                             | 20             |
| 10. | <u>PRINTING AND REPRODUCTION</u>             |                             |                |
|     |  | <u>2000</u>                 | _____          |
|     | Sub Total                                    | 2000                        |                |
| 11. | <u>OTHER CONTRACTUAL SERVICES</u>            |                             |                |
|     | None   |                             |                |
| 12. | <u>CONSTRUCTION, RENOVATION, ALTERATIONS</u> |                             |                |
|     | None   |                             |                |

B. BUDGET SUMMARY

| <u>Category</u>           | <u>Project Requirements</u> |                |
|---------------------------|-----------------------------|----------------|
|                           | <u>TAKA</u>                 | <u>DOLLARS</u> |
| 1. Personnel              | 2000                        | 1,737.50       |
| 2. Supplies               | 200                         | 100            |
| 3. Equipment              |                             |                |
| 4. Hospitalization        |                             |                |
| 5. Outpatients            |                             |                |
| 6. CRL Transport          | 840                         |                |
| 7. Travel Persons         |                             |                |
| 8. Transportation Things  |                             |                |
| 9. Rent/Communication     |                             | 20             |
| 10. Printing/Reproduction | 2000                        |                |
| 11. Contractual Service   |                             |                |
| 12. Construction          |                             |                |
|                           | <hr/>                       | <hr/>          |
|                           | Total: 5040                 | 1,857.50       |
|                           | Total \$                    | 2,193.50       |
|                           | Total to CRL: \$            | 456            |

Conversion Rate \$ 1 : Tr. 15



### ABSTRACT SUMMARY

1. Fifty lactating women will be required for this study since breast milk samples will be collected.
2. No risk is associated with obtaining breast milk. In fact it is the custom in Bangladesh for mothers to express the colostrum and discard it. We would be collecting the milk which would otherwise be discarded.
3. Not applicable.
4. Names, addresses will not be obtained. Patients will be identified by a code number and there will be no link back to the patient.
5. Oral consent will be obtained. Because of the absence of any risk, written consent is not warranted. The study will be described to the women as shown on the enclosed form.
6. The enclosed questionnaire will be filled out.
7. There are no risks. The individuals will not benefit from the study. Society may benefit through eventual control of diarrhea diseases which requires studies to gain a better understanding of the epidemiology and immunology of the diseases.
8. The study requires milk samples only.

QUESTIONNAIRE FOR BREAST MILK STUDY

Date of collection \_\_\_\_\_

Collected by \_\_\_\_\_

Milk Sample number D \_\_\_\_\_

Date of delivery \_\_\_\_\_

How many children does the mother have now \_\_\_\_\_

Does the mother usually breast-feed her children \_\_\_\_\_

How old will the baby be when she stops breast-feeding \_\_\_\_\_

Did she have an episode of severe diarrhea during her pregnancy \_\_\_\_\_

If yes, describe \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

Was she admitted to the Cholera Hospital \_\_\_\_\_ When \_\_\_\_\_

### ORAL CONSENT INFORMATION

Because of the absence of risks, and the nature of the study, consent will be given orally. With each individual, the investigator or the field worker will explain the study in the following manner.

"The Cholera Research Hospital is doing research in diarrheal diseases. One study, which we would invite you to participate in, is one in which the amount of antibody to certain diarrheal diseases (protective substance) will be measured in breast milk. This will help us gain a better understanding of these diseases. If you agree to participate in the study, we will ask you some questions about your health, about your plans for breast feeding your baby and we will take a small amount of milk to be tested for the level of antibody.

There are no risks to participating in the study. You do not have to participate and your care in this hospital will not be changed or altered in any way because of your decision regarding the study. All of this information will be kept strictly confidential."

যেহেতু ওই শ্রমীকৃত্য বুকি নেই, তমোখিক  
সম্মতি দেয়া হয়। প্রাতক কৃষ্টিয় মাথ  
সাবধক অথবা ফিল্ড ওয়ারকাৰ (field worker)  
শ্রমীকৃত্যে বিখ্য নিম্ননিখিত ডাব ব্রুধা কৰােন,

"কালনা সাবধনা অমমাতান উদযায়- স-কাম্ব  
বোজা নিখে সাবধনা চালাকেন, এ শ্রমীকৃত্য  
অমময় আকমাক অংশ ব্রহনয় জয় আকম্বন  
জানকি ও হাঙ্ক বুকয় দুৰ্ব কতভুলো নিদ্রিষ্  
প্রকাৰ উদযায় বোজাও প্রকির্ষক কি শ্রমিমান  
আছে ও নিৰ্ময় কৰা হয়, যদি আকমি ও -  
শ্রমীকৃত্য অংশ ব্রহন কৰাত সম্মত হন ও হলে  
আকময় সূক্ষ ও আকময় বাধাক বুকয় দুৰ্ব  
খাণ্ডয়লাক ব্রময় আকময় সম্মত সম্মত  
কতভুলো প্রমা কিঙ্কম কৰা হয় ওয়  
সম্মত শ্রমিমান দুৰ্ব শ্রমীকৃত্য জয় দেয়া হয়।

ওই শ্রমীকৃত্য অংশ ব্রহন কোন প্রকাৰ বুকি  
নেই। আকমাক কোন কিহাও অংশ ব্রহন  
কৰাত হবনা অথবা শ্রমীকৃত্য অংশ  
ব্রহনয় জয় অমমাতান আকময় দেয়া-সূক্ষ  
কোন প্রকাৰ শ্রমিমান হবনা, ও মকেন  
তথ্য প্রকাৰ আকম বাধা হয়।"