Attachment 1.

library

Date 15 May, 1989

T6/5

ETHICAL REVIEW COMMITTEE, ICDDR,B.
Dhaka 12.2

Principal Investigators Dr. Saul Tzipori
Dr. D. Mahalanabis
Dr. R. Beckels

Trainee Investigator (if any) _____

Application No. ~~89-000~~ 89-003

Supporting Agency (if Non-ICDDR,B) _____

Title of Study: Evaluation of hyperimmune bovine colostrum in the treatment of (a) rotavirus diarrhoea in infants and (b) Shigella disease in children

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 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 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.

(PTO)

We 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

AUG 15 1989

Trainee

SECTION I: RESEARCH PROTOCOL

1. Title: Evaluation of hyperimmine bovine colostrum in the treatment of (a) rotavirus diarrhoea in infants and (b) Shigella disease in children.

2. Principal Investigator(s): Dr. Saul Tzipori
   Dr. D. Mahalanabis
   Dr. R. Eeckels

   Co-investigator(s): Dr. Hasan Ashraf
   Dr. Amal Mitra

3. Starting Date: July, 1989

4. Completion Date: June, 1991

5. Total Direct Cost: US$ 27,000

6. Name of the Scientific Division(s) approving new protocol: Clinical Sciences Division

Signature of the Division Head: Dr. D. Mahalanabis

Date: 16th May 1989

89-002
16/5

Evaluation of hyperimmune bovine colostrum in the treatment of (a) rotavirus diarrhoea in infants and (b) Shigella disease in children

Principal investigators: Dr. Saul Tzipori
Dr. D. Mahalanabis
Dr. R. Eeckels

Co-Investigators: Dr. Hasan Ashraf
Dr. Amal Mitra

A&B. INTRODUCTION

1. Objectives, rationale and Specific Aims

General Objectives

To evaluate the therapeutic efficacy of a hyperimmune bovine colostrum (HBC) product specifically prepared to contain very high titres of antibody against the offending pathogen. The product will be evaluated in two parts i.e. controlled clinical trial A) in infants with proven rotavirus diarrhoea and B) in children with bloody diarrhoea due to shigella species.

Rationale

1. Trial of HBC in rotavirus diarrhoea

- Rotavirus is an important cause of severe diarrhoeaa in infants and small children, often requiring hospitalisation;

- Existing treatment of rotavirus diarrhoea includes a) replacement of fluid and electrolyte loss due to diarrhoea and vomiting, mainly with oral rehydration therapy (ORT), and b) appropriate feeding during and after diarrhoea to minimise nutritional consequences;

- ORT does not reduce the severity and duration of diarrhoea and therefore, demand for useless and often harmful drugs is very high;

- Results of clinical trials with various antidiarrhoea drugs have been disappointing; it is therefore important to evaluate new approaches to develop antidiarrhoea medicines;

- Use of hyperimmune bovine colostrum is an immunological approach to treat enteric infections with a colostrum containing a very high titre of antibodies against the pathogen; similar preparations have been successfully used to treat intractable diarrhoea due to cryptosporidiosis in an immunocompromised patient.

- Bovine colostrum is a highly nutritious food product and should have no side effects.

2. Trial of HBC in Shigellosis:

- In developing countries and particularly in Bangladesh shigellosis is a leading cause of morbidity and mortality in children. It has been estimated that approximately 0.7 to 0.8 million deaths occur in children under 5 from dysentery each year in the developing countries.

- Antibiotics are the cornerstone of treatment for shigellosis. Eradication of these invasive organisms shortens the clinical illness substantially.

- Rapid emergence of resistance of Shigella species against antibiotics is a serious and unsolved problem. It is therefore a high priority to evaluate novel approaches to treat shigellosis.

- HBC containing very high titres of antibody against Shigella dys.1 and Shigella Flex, if found effective should be a valuable therapeutic agent

with no side effects. Furthermore, these organisms are not likely to develop resistance against HBC.

Specific Aims

1. To determine whether hyperimmune bovine colostrum with a high titre of antibody against 4 serotypes of human rotavirus antigens (HBC-S) reduces the severity and duration of rotavirus diarrhoea in infants and children aged 6 monmths to 2 years (Part A of the study).

2. To determine whether hyperimmune bovine colostrum with a high titre of antibody against antigens of two major Shigellae species i.e. Shigella dys.1 and Shigella Flexneri (HBC-S) reduces the severity and duration of disease in children aged 1 year to 7 years suffering from acute shigellosis due to Shigella dys.1 and Shigella Flexneri.

Background

Human bovine colostrum was used successfully as an antimicrobial agent on human volunteers challenged with enterotoxigenic E.coli; the aim of the study

was to evaluate such an approach for providing prophylaxis for travellers diarrhoea (1). It was also demonstrated to be an effective treatment against persistent diarrhoea due to cryptosporideosis in immune deficient individuals (2,3). Animal studies suggest that orally administered virus neutralizing milk and blood serum provides local passive immune protection against experimental rotavirus infection (4,5,6).

Favourable results were also obtained in treating rotavirus infection in human and animals using colostral and human blood serum antibodies administered orally (7-12).

The use of HBC is an attractive option in the management of enteric infections against which there is no specific treatment e.g. rotaviruses. HBC is also particularly relevant to the treatment of shigellosis in which the only effective treatment is antimicrobial agents. As stated earlier rapid emergence of resistance of shigella species against antibiotics is a serious and unsolved problem.

Colostrum is available in large quantities in high milk yielding cows. Colostrum is not used for human consumption and can be made available for

this purpose fairly cheaply once a system has been established for its production and processing. The HBC may be used for treatment either as whole milk or as separated and freeze-dried immunoglobulin or as whey. The major immunoglobulin isotype of bovine cholostrum is IgG 1, followed IgG 2, IgA and Igm. Unlike monogastric mammals in which IgA plays a major role, in ruminants IgG 1 provides the principle lactogenic immunity to the new born which, like IgA, is somewhat resistant to acid and proteolytic enzymes.

Production of HBC

The method of producing hyperimmune bovine colostrum against specific microbes has been already developed by one of us (Tzipori 2,3). Briefly ten to twelve weeks prior to parturition pregnant cows in nominated dairy herds were vaccinated with antigens prepared from enteric pathogens. Six cows were used for each of the pathogens which included rotavirus and shigella species. Six cows were used to obtain normal milk for control.

A. Production of HBC against Rotavirus:

Pregnant cows were vaccinated with each of the four cell culture adapted human rotavirus serotypes. Viral antigen (titre 10^8) was mixed with a equal volume of fruend's incomplete adjuvant (total volume 8 ml) which was given parenterally followed two weeks later by intramamary infusion of the same preparation via the teat canal. A third injection was given two weeks later (4-6 weeks prior to calving) in the same site as the first injection. After calving the colostrum was collected during the first two days which yielded 15 to 20 litres of antibody-rich colostrum per cow. Colostrum was collected for the first two days only. It was then frozen at -20^{o}C in five litre containers. Representative samples for testing sterility and amount of specificity of Ig was taken before freezing each container. The ingredients present in bovine colostrum are included in Appendix A. The amount and specificity of Ig was tested at the Royal Childrens Hospital in Melbourne to assure that only products with high titres were included in the products.

B. Production of HBC against Shigella species:

A mixture of heat killed (60 °C over 30 mins) and formalin killed (0.2%) of 10^{11} organisms (shigella dysentery or shigella flexneri) were mixed in equal volume with adjuvant (total of 8ml) which was administered to cows as described for rotavirus. The procedure for inactivation of bacteria was the same as that described for cholera vaccine (13).

C. METHODS OF PROCEDURE

Part A of the Study

Controlled trial of HBC-R in infants and small children with rotavirus diarrhoea:

A double blind randomised trial design will be used. The study patients will be treated according to the standard treatment routine at ICDDR,B; in addition they will receive HBC-R. Patients in the control group will be similarly treated but instead of HBC-R they will receive the same amount of bovine cholostrum from cows not specifically immunised with rotavirus antigen.

1. Inclusion criteria:

a. males aged 6 months to 24 months;

b. history of acute watery diarrhoea of 3 days or less and had 4 or more liquid stools over 24 hours prior to admission;

c. positive stool test for rotavirus antigen using a rapid diagnostic procedure (e.g. latex agglutination test);

d. the child has signs of dehydration.

2. Exclusion Criteria

a. systemic infection requiring prompt antibiotic treatment;

b. clinically apparent severe marasmus or kwashiorkor;

c. history of bloody diarrhoea.

3. Response Variables

Major:

a. Total stool output in g/kg of body weight, from the time of initiation of treatment with HBC-R until the end of diarrhoea;

b. Duration of diarrhoea, in hours, after randomisation;

Secondary:

c. Stool output, in g/kg/, during the first 24 hours of treatment with HBC-R.

d. Intake of ORS solution from start of HBC-R treatment until diarrhoea stops.

e. Vomiting - number and duration during the treatment with HBC-R.

4. Sample Size

The present study is largely exploratory (i.e. HBC under evaluation is not the definitive product for real life use and the results will be used for further development of the product). We are only interested if the product improves diarrhoea duration and severity. Therefore a one-sided test to detect improvement should be adequate for this study. In a group of infants with rotavirus diarrhoea (Sack et al, The Lancet 1978, Vol.II, 280-283) the mean duration of diarrhoea after admission to the hospital was 57 hours (SD=26). For this treatment to be reduced by at least 30%. To detect a difference of this magnitude (=.05, =0.2) the sample size is 29 in each group i.e. 58 for deviated course or false positive initial rotavirus diagnosis we increase it by 10% which brings the sample size to 64 patients.

5. Randomisation

The patients in the study group will receive hyperimmune bovine colostrum (HBC-R) from cows immunised with rotavirus antigen from 4 serotypes of human rotavirus (for methods of preparation see below). Patients in the control group will receive the same amount of bovine colostrum (BC) from cows not immunised with rotavirus antigen. Colostrum preparations will be dispensed in indentical containers. A master randomisation chart will be prepared by an appropriately trained person who is not connected with the study using random permuted blocks (to equalise the number of patients in the two groups after short intervals). Bottles containing HBC-R and BC will be arranged in a sequence that corresponds to the randomisation chart and serialy numbered. The serial number of the bottles will correspond to the serial number of the patients enrolled into the study.

6. Patient Recruitment

Infants and children presenting at the treatment centre with a history of watery diarrhoea of 3 days or less and 4 or more liquid stools during previous 24 hours will be evaluated and if they fulfill the remaining

inclusion and exclusion criteria will be admitted to the study. A rapid test for rotavirus (latex agglutination) antigen will be used to ascertain its presence within 4 hours of admission and if positive will be included in the study. The diagnosis will be confirmed by appropriate ELISA test.

7. Informed Consent

If the patient is found eligible for inclusion into the study an informed consent will be obtained. The consent form will be administered by one of the investigators and then will be witnessed by another staff member.

8. Case Management

Patients will receive routine care which includes a) replacement of fluid and electrolytes mainly with ORT supported by IV: treatment for those who need it; b) unrestricted breastfeeding of those still breastfed during rehydration and maintenance therapy, and c) formula milk for nonbreastfed infants starting after initial rehydration period and d) semisolid and solid food appropriate for age during maintenance fluid therapy.

Bovine colostrum will be given as drinks from the bottle assigned to the study patient, 100ml every 6

hours for 3 days starting soon after the patient has been assigned to the study.

9. Summary of Procedures

On Admission

- stool/rectal swab will be tested for rotavirus antigen (immediately by rapid method and later on by ELISA) and cultured for detection of toxigenic E.coli, V.cholerae, C.jejuni and shigellae species;
- stool sample will be frozen daily for testing for rota antibodies and antigen.
- blood for microhematocrit, serum, totai solids and electrolytes will be taken (1 ml)
- intake and output measurements will be instituted using urine bags and cholera cots.

During course of illness

- patients will be evaluated every 6 hours and intake/output measurements will be summarised from tally sheets;
- patients will be weighed and physical examination findings will be recorded and time of

cessation of diarrhoea will be noted.

- a second blood sample will be taken at 48 hours for Hct, plasma total solids and electrolytes (1ml).

10. Definitions

1. Duration of diarrhoea: the time in hours from initiation of the study treatment until passage of the last liquid or semiliquid stool prior to two formed/soft stools or prior to 12 hours during which no stool is passed.

2. Stool output: The weight of stool in g/kg of admission body weight expressed per time period (e.g. per 24 hours or for the entire duration of diarrhoea)

3. ORS and plain water intake: The total of ORS or plain water taken in ml/kg of admission body weight expressed per time period (e.g. per 24 hours or for the entire duration of diarrhoea)

11. Withdrawals from the study

1. Non-compliance of the subject, either because the patient leaves the hospital before the end of

the study or because the patient requires unscheduled treatment for a serious illness.

2. Treatment of Withdrawals during analysis: Results of all randomised patients will be included in the analysis of the study. Data from patients withdrawn will be included upto the time of withdrawal. A supplemental analysis in which such patients are excluded will also be made.

12. Study Schedule

1. Procurement of supplies and standardisation of methods and development/testing of data forms and recruitment and training of personnel - 1 mohth.

2. Conduct of the trial - 12 months

3. Analysis of data and preparing report - 2 months.

13. Data Analysis

The study groups will be compared for all the variables prior to intervention (i.e. initiation of bovine colostrum). Major outcome variables will be compared after evaluating their

descriptive statistics for distribution etc. The quantitative outcome measures will be compared using Student's 'T' Test on primary data or, after appropriate transformation when indicated, and also by an equivalent nonparametric test e.g. Mann Whitney U test. Dichotomous outcome measures will be compared by χ^2 test or Fisher's exact test as appropriate.

Controlled clinical trial of HBC-S in children aged 1 to 7 years with shigellosis

A double blind randomised trial design will be used. The study patients will be treated according to the standard treatment routine at ICDDR,B; in addition they will receive HBC-S. Patients in the control group will be similarly treated but, instead of HBC-S they will receive the same amount of colostrum from cows not specifically immunised with shigella antigen.

1. Inclusion criteria:

   a. Patients aged 1 to 7 years of either sex with a a history of bloody diarrhoea of less than 72 hours duration.

   b. Stool microscopy shows more than 20 pus cells per high power field.

2. Exclusion Criteria:

   a. Patients who took drugs during current illness which are potentially effective against Shigella disease (i.e., Ampicillin, Nalidixic Acid, Pivmecillinam, TMP-SMX);

b. Patients with additional and obvious systemic illness (e.g. pneumonia, meningitis);

c. Severely malnourished patients.

3. Response Variables

a. Clinical "cure" rate on day 3; cure being defined by absence of watery diarrhoea and number of bowel motions equal to or less than 4 in previous 24 hours.

b. Mean stool frequency on each day of treatment;

c. Duration of abdominal pain/tenderness by eight-hourly evaluation;

4. Sample Size Estimate

a. Sample size based on clinical "cure" rate on day 3 - Data from a previous clinical trial of nalidixic acid and ampicillin in shigellosis in children showed that on day 3 patients on ampicillin (to which Shigella species were sensitive) had mean stool frequency of 8.8 with an SD of 6.1. We expect that on new treatment (i.e. Nalidixic acid plus HBC-S) the mean stool frequency will be reduced to 4 or less. The calculated sample size to detect this

degree of difference with 95% confidence (= 0.05 and 80% power (=0.2) would be 52 patients. Assuming that 70% of the patients will be positive for Shigella isolates in the stool the sample size would be 74 patients. With an anticipated drop out rate of 6 per group - the total sample size is 100 patients.

5. Randomisation

The patients in the study group will receive hyperimmune bovine colostrum (HBC-S) from cows immunised with Shigella antigen from 2 Shigella sp. (for methods of preparation see below). Patients in the control group will receive the same amount of bovine colostrum (BC) from cows not immunised with Shigella antigen. Colostrum preparations will be dispensed in identical containers. A master randomisation chart will be prepared by an appropriately trained person who is not connected with the study using random permuted blocks (to equalise the number of patients in the two groups after short intervals). Bottles containing HBC-R and BC will be arranged in a sequence that corresponds to the randomisation chart and serially numbered. The serial number of the bottles will correspond to the serial number of the patients enrolled into the study.

6. Patient Recruitment

Children presenting at the treatment centre with a history of bloody diarrhoea of 3 days or less will be evaluated and if they fulfill the remaining inclusion criteria, will be admitted to the study.

7. Informed Consent

If the patient is found eligible for inclusion into the study an informed consent will be obtained. The consent form will be administered by one of the investigators and then will be witnessed by another staff member.

8. Case Management

Patients will receive routine treatment for similar patients in the hospital which include a) antibiotic treatment appropriate for shigellosis patients i.e. Nalidixic acud, b) fluid therapy as needed, c) appropriate diet for age. In addition they will receive bovine colostrum according to the randomisation schedule described earlier.

9. Summary of Procedures

a. On admission

- History and physical examination findings (including nutrition anthropometry) will be recorded on a pretested form.

- Stool/rectal swab on admission will be tested for Shigella species , C. Jejuni, Salmonellae, V. Cholerae Aeromonas hydrophila, plessimonas shigellosis, and rotavirus, and stool microscopy for pus cells and parasites; daily stool samples will be frozen for shiga toxin and antibody measurements.

- Blood test for microhematocrit, plasma total solids, electrolytes, WBC total and differential count.

10. Withdrawals from the study

Non-compliance of the subject, either because the patient leaves the hospital before the end of the

study or because the patient requires unscheduled treatment for a serious interim illness.

Treatment of withdrawals during analysis:
Results of all randomised patients will be included in the analysis of the study; data from patients withdrawn from the study will be included upto the time of withdrawal; a supplemental analysis in which such patients are excluded will also be made.

11. Study Schedule

Procurement of supplies and standardisation of methods and development/testing of data forms and recruitment and training of personnel - 1 month.

Conduct of trial - 18 months

Analysis of data and writing of report - 3 months.

BUDGET SUMMARY

1. PERSONNEL	% Full time	Expenditure in US$
Senior Health Assistant (1)	100%	4226
Hospital Attendant (4)	100%	2600
Technician (1)	30%	1130
Sub Total		7956

2. OPERATING EXPENSES	
- Supplies, chemicals, glassware, other misc. items	2000
- Small equipment	3000
- Diapers, urine bags, drugs etc.	2000
- Data Management/Analysis	1000
- Laboratory tests (Diagnostic microbiology, clinicakl chemistry, processing and freezing samples, serotyping of Rotavirus, Shigatoxin and its antibody determination etc.)	10000
- Communication, utilities, printing, publication, xeroxing, telex, medical illustrations, file, file cabinets etc.	1000
Total	26956

REFERENCES

1. Tacket et al. 1988. protection by milk immuglobulin concentrate against oral challenge with enterotoxigenic E.coli. New Engl. J. Med. 318:1240-1243.

2. Tzipori et al. 1986. Remission of diarrhoea due to cryptosporidiosis in an immunodeficient child treated with hyperimmune bovine colostrum. Br. Med. J. 293:1276-1277.

3. Tzipori et al. 1987. Chronic cryptosporidal diarrhoea and hyperimmune cow colostrum. Lancet; ii:344-345.

4. Bartz CR, Conklin RH, Tunstall CB, Steele JH. 1980. Prevention of murine rotavirus infection with chicken egg yolk immunoglobulins. J. Infect. Dis; 142:439-41.

5. Bridger JC, Brown JF. 1981. Development of immunity to porcine rotavirus in piglets protected from disease by bovine colostrum. Infect Immun; 31:906-10.

6. Saif LJ, Redman DR, Smith KL, Theil KW. 1983. Passive immunity to bovine rotavirus in newborn calves fed colostrum supplements from immunized or nonimmunized cows. Infect Immun; 41:1118-31.

7. Hilpert H, Brussow H, Mietens C, Sidoti J, et al. 1987 Use of bovine milk concentrate containing antibody to rotavirus to treat rotavirus gastroenteritis in infants. Journal of Infect Dis.,156:158-166.

8. Offit PA, Clark HF. Protection against rotavirus-induced gastroenteritis in a murine model by passively acquired gastrointestinal but not circulating antibodies. J Virol 1985; 54:58-64.

9. Bishop RF, Cameron DJS, Veenstra AA, Barnes GL. Diarrhoea and rotavirus infection associated with differing regiments for postnatal care of newborn babies. J. Clin. Microbiol 1979; 9:525-9.

10. Barnes GL, Doyle LW, Hewson PH, Knoches AML, McLean JA, et al. A randomised trial of oral gammaglobulin in low-birth-weight infants infected with rotavirus. Lancet 1982; 1:1371-3.

11. Ebina T, Sato A, Umezu K, Ishida N, Ohayama S, et al. Prevention of rotavirus infection in low colostrum containing antibody against human rotavirus. Lancet 1983; 2:1029-30.

12. Hilpert H, Gerber H, Amster H, Pahud JJ, et al. Bovine milk immunoglobulins (Ig), their possible utilization in industrially prepared infant's milk formulae. In: Hambraeus L, Hanson LA, McFarlene H: eds. Food and Immunology. Stockholm: Swedish Nutrition Foundation, 1977:182.

13. Clements J et al. 1987. Subunit-whole cell and whole cell oral vaccines against cholera: studies on reactogenecity and immunogenecity. J. Infect. Dis., 155:79-85.

Appendix A

Composition of the Colostrum

Constituents	Colostrum			Normal milk
	0 hr	12hr	24hr	
Total solids (%)	24.75	20.71	17.09	12.86
Lactose (%)	3.10	3.10	3.10	4.60
Ash(%)	1.12	1.04	0.96	0.72
Choline (PPM)	370	320	690	130
Protein (%)	11.35	9.60	7.07	3.25
Casein (%)	5.2	5.31	5.2	3.1
Albumin (%)	1.5	1.2	1.0	1.5
Immunoglobulin(mg/ml)	38.23	32.22	21.52	-

THE JOHNS HOPKINS UNIVERSITY
SCHOOL OF HYGIENE AND PUBLIC HEALTH

Department of International Health

Division of Geographic Medicine

May 2, 1989

Dr. Dilip Mahalanabis
1 CDDR, B
GPO Box 128
Dhaka 2, Bangladesh

Dear Dilip:

Please find enclosed my comments on the proposal entitled, "Evaluation of hyperimmune bovine colostrum in the treatment of (a) rotavirus diarrhoea in infants and (b) Shigella disease in children".

I am sending a copy of my comments by FAX. The original has been mailed to you.

Good luck with the study.

Sincerely,

Mathuram Santosham, M.D., M.P.H.
Associate Professor, International Health
Division of Geographic Medicine

MS:ajr

Enclosure

The Johns Hopkins University • 615 North Wolfe Street • Baltimore, Maryland 21205
Telex: 7102340022 PUB HYG BAL • Cable Address: PUB HYG

By: M. Santosham

RE: Proposal entitled "Evaluation of hyperimmune bovine colostrum in the treatment of (a) rotavirus diarrhoea in infants and (b) Shigella disease in children"

Principal investigators: Dr. Saul Tzipori
Dr. D. Mahalanabis
Dr. R. Eeckels

Co-Investigators: Dr. Hasan Ashraf
Dr. Amal Mitra

This proposal is well written and the hypothesis has been well defined. The information that can be potentially gained from this study will provide valuable information for the treatment of rotavirus and Shigella infections. I have the following minor suggestions for amending the protocol that the principal investigators may want to consider:

1. Inclusion criteria - I would suggest that infants with diarrhea of less than two days duration rather than three days duration should be included. The reason for this suggestion is that rotavirus diarrhea often begins to resolve after 48 hours. Therefore, regardless of the treatment group, infants who have had diarrhea for more than a 48 hour period are likely to resolve within the next 24 hours, which would reduce the chances of demonstrating any differences between the two groups.

2. Sample size calculations - In the sample size calculations it is assumed that the efficacy of the test product in preventing diarrhea will be similar for the four serotypes of rotavirus. It is quite likely that protection will be more effective for certain serotypes compared to others. Therefore, they may want to make some corrections on their sample size calculations to account for this.

3. Case management: feeding regimen during the treatment period - Since breastfeeding itself may reduce the duration of diarrhea, I would suggest that the randomization should be stratified for breastfeeding. It is also stated that food, (semi-solid and solid food appropriate for age during maintenance fluid therapy) will be given. I would suggest that

the feeding regimen should be standardized in some form so that all infants that are non-breastfed are receiving the same nutritional and calorie intake in order to allow comparability between the two groups. It is now increasingly evident that introducing different kinds of food may modify the severity and duration of diarrhea.

4. What provision has been made for infants who refuse to accept the bovine colostrum or infants that drink only a portion of the colostrum that is offered?

5. Page 18 2.c. Severely malnourished patients should be defined.

6. Page 18 3.a. Response variables - It is not clear why the authors chose "cure" rate on day three as an end point. It would be just as interesting to know what percent of infants are cured on days one and two, after the treatment is instituted.

7. Page 18 3.c. Duration of abdominal pain/tenderness is very difficult to measure in infants under two years of age.

8. Are there any failure criteria? Will infants who become dehydrated and require hospitalization for IV therapy also be included in this analysis? How will patients who continue to have diarrhea beyond a one week period be handled for analysis? Will they be considered to be study failures?

Review of Protocol entitled "Evaluation of hyperimmune bovine colostrum in the treatment of (a) rotavirus diarrhoea in infants and (b) Shigella disease in Children" Investigators: Drs. Tzipori, Mahalanabis, Eeckels.

The study plans to see the effect of administration of specific hyperimmune bovine colostrum to children with (duration of diarrhoea less than 3 days and with no prior treatment) from whom rotavirus or shigellae have been identified as a novel method of therapy.

The method of production of the hyperimmune bovine colostrum has been validated previously. The details of sample size were not available at the time of review.

How a rapid diagnosis of the etiological agent would be made is not mentioned. Since randomisation is dependent on the etiology, given the available techniques at Dhaka, there is likely to be a delay of 24 hours before the child can be assigned to a treatment group. This should invalidate the design of the study as the child would in general have responded to treatment by then.

It is unlikely that therapy with hyperimmune bovine colostrum would have a major public ~~control~~. However it could contribute significantly to understanding intestinal immunity if combined with recovery of the pathogens,

preferably from the small intestinal lumen, in the case of rotavirus and from the stool in the case of Shigellae after various durations of colostrom therapy to determine whether these are viable, invasive or infective inappropriate in vitro assays. If this can be done the study will be valuable in helping us to understand protective mechanisms.

Prof. V.I. Mathan

March 17, 1989