achment 1.
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ETHICAL REVIEW COMMITTEE, ICDDR,B.

Date 05.05.96
15/5/96
(6)

ncipal Investigator Dr. S.A. Sarker
lication No. 96-009
le of Study Evaluation of Chicken Yolk Immunoglobulin (IgY) in the treatment of diarrhoea due to rotavirus in children

Trainee Investigator (if any) _____
Supporting Agency (if Non-ICDDR,B) _____
Project status:
(✓) New Study
( ) Continuation with change
( ) No change (do not fill out rest of form)

cle 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.

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

S A Sarker
Principal Investigator

Trainee

ENTERED 2 1 JUN 1996

Title: **Evaluation of Chicken Egg Yolk Immunoglobulin (IgY) in the treatment of diarrhoea due to rotavirus in children**

Principal Investigator	:	Dr. S. A. Sarker
Collaborating Investigators:		Dr. T. H. Casswall Dr. L. Hammarström Karolinska Institute, Sweden Dr. K. Mujo Dr. J. L. Raj Taiyo Kagaku Co. LTD, Japan
Co-Investigators	:	Dr. G. Fuchs Dr. P. K. Bardhan Dr. N. H. Alam Dr. M. J. Albert Ms. L. Unicomb
Starting date:		June 1 st 1996
Completion date:		15-18 months after starting
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Signature of Division Director
Clinical Sciences Division

Date: May 5 1996

Abstract Summary

Use of oral immunoglobulin therapy in man using antibodies from both humans and animal sources is gaining popularity to treat enteric infections. Hyperimmune bovine colostrum (HBC), containing high levels of certain antibodies against human rotavirus, enterotoxigenic *Escherichia coli* and *Helicobacter pylori* has been used as prophylaxis and as therapy with encouraging results in humans. The prophylactic and therapeutic effect of egg yolk immunoglobulin from hens immunized against human rotavirus, has been studied in animals but not so far in humans. However there is reason to believe, based on the above animal studies, and on studies with HBC, that chicken antibodies may also be effective in human therapy. To evaluate the therapeutic efficacy of such specially prepared egg yolk immunoglobulin in rotavirus diarrhoea in children, a randomised, placebo-controlled double-blind clinical trial is proposed. Ninety male children (6-24 months) with rotavirus diarrhoea will be studied. The children will be randomly assigned to Chicken Egg Yolk immunoglobulin (IgY), from immunized hens or IgY obtained from non-immunised hens (=placebo). They will be treated in hospital with 10 g of the assigned immunoglobulin in four divided doses for 4 days. Stool output (ml/kg/day), frequency (no/day) and clearance of rotavirus in stool will be compared between children receiving active substance or placebo.

Objectives

To evaluate the therapeutic efficacy of chicken egg yolk immunoglobulin product specially prepared to contain very high titre of antibody against rotavirus (IgY-R). The product will be evaluated by a controlled clinical trial in infants with proven rotavirus diarrhoea.

Background

Immunotherapy in enteric infections - Human antibodies

The concept of immunotherapy in enteric infections has recently gained acceptance and is based partly on the observation that patients with various forms of antibody deficiency syndromes exhibit an increased frequency of gastrointestinal infections, suggesting that local immunity in the gut in the form of antibodies may play a major role in the local mucosal defense. Breast milk which contains secretory IgA has been shown to protect against diarrhoea in infants (Cunningham et al 1977, Larsen et al 1978, Glass et al 1983) suggesting an important role of this antibody in mucosal defense. A prophylactic effect in prematurely born infants against necrotizing enterocolitis of orally fed IgA from serum (Eibi et al 1988), a disease of presumed infectious etiology has also been shown recently and this preparation has also been shown to exhibit a therapeutic benefit in *Clostridium difficile* induced diarrhoea (Tjellstrom et al 1993). Animal studies suggest that orally administered virus neutralizing milk and serum provide local passive immune protection against experimental rotavirus infection (Bartz et al 1980, Bridger et al 1981, Saif et al 1983). Favorable results have also been obtained in treating rotavirus infection in humans and animals using colostral and human serum antibodies administered orally (Hilpert et al 1987, Brussow et al 1987, Offit et al 1985, Bishop et al 1979, Barnes et al 1982, Ebina et al 1983). A field trial with a milk formula supplemented with 1% immunoglobulin concentrate directed against Enteropathogenic *Escherichia coli* (EPEC) and rotavirus infection however, has failed to prevent EPEC and rotavirus diarrhoea (Brunser et al 1992). Failure to get a positive effect on diarrhoeal disease may be related to the low concentration of immunoglobulin used in this studies.

Secretory IgA is endowed with unique properties which are due to its divalent form (two antibodies joined by J chain and a secretory component) which render it resistant to proteolysis in the gastrointestinal tract. From a theoretical point of view, human IgG due to its monovalent form could therefore be less effective in gastrointestinal infections. However, it has been estimated that 25% of orally administered IgG survives the passage through the gastrointestinal tract in patients with diarrhoea (Losonsky et al 1985, Blum et al 1981). Subsequently, human IgG has been tried both as a prophylaxis (Tutshka et al 1987, Barnes et al 1992) and therapy in rotavirus infection in low birth weight infants (Barnes et al 1982), cryptosporidial infection (Guarino et al 1991) and non specific diarrhoea in children (Melamed et al

1991). But the titre against most pathogens is low in human gammaglobulin preparations and vaccination of volunteers for establishing hyperimmune preparations is not feasible.
Because of the high cost of human preparations, alternative sources of antibodies, as for example bovine colostrum, or chicken egg yolk have therefore been sought by researchers for the last few years.

Bovine antibodies and hyperimmune cow colostrum

Immunoglobulin from various species have been used for prophylactic and therapy against infection for more than a hundred years.
Bovine colostrum is available in large quantities in high milk yielding cows. The antibodies are transported to the milk in cows and are present in high concentration in colostrum where it protects the calves from infections during the neonatal period. Promising results have been reported using colostrum from non-immunized cows (Lactobin) in reducing the severity of diarrhoea in AIDS patients suffering from non-specific diarrhoea (Rump et al 1992). Antibodies against intestinal pathogens such as *Salmonella* (DO and BO antigens), *Shigella flexneri* (LPS), *Yersinia enterocolitica*, rotavirus, *Helicobacter pylori*, *Campylobacter jejuni* and ST and LT toxins from *E-coli* are found in the colostrum of cows. Although colostrum from non-immunized cows confer therapeutic benefit against some enteric pathogens, the major limitations of using it, derives from the low titer of immunoglobulin against pathogens.

If the cow is vaccinated against different pathogens during pregnancy, one will have a colostrum which contains very high levels of antibodies against these microbial antigens. It may be used for therapy either as whole milk or separated and freeze-dried immunoglobulin or as whey.

Hyperimmune bovine colostrum (HBC) contains for example titre with 100-1000 times higher neutralization capacity against rotavirus and thus, HBC is an attractive option to treat enteric infections (Brussow et al 1987, Hilpert et al 1987). HBC has also been obtained from cows immunized with *Escherichia coli* (Mittens et al 1989, Tacket et al 1988) and *Vibrio cholerae* (Boesman 1989). The product has been demonstrated to protect against challenge with live *E. coli* (Tacket et al 1988) and *Shigella flexneri* (Tacket et al 1992) in human volunteers.

Trials with HBC have also reported early recovery from cryptosporidial diarrhoea in immunodeficient children (Tzipori et al 1986 and 1987), in patients with acquired immunodeficiency syndrome or human immunodeficiency viral infection and immunocompromised patients with chronic *Campylobacter* infection (Hammarstrom et al 1993).

HBC raised against rotavirus has been shown to prevent rotavirus infection in hospitalized children (Davidson et al 1989) and children living in orphanage (Ebina et al 1985). When, however, the antibodies from colostrum of cows, immunized against *rotavirus* and *E-coli*, was diluted (1%) in commercial milk formula, no significant protective effect was seen in a double-blind placebo-controlled field trial in Chile (Brunser et al 1992). Clinical studies to evaluate therapeutic effectiveness of HBC in infantile diarrhoea are therefore scanty. Limited studies with HBC raised against rotavirus have demonstrated that it reduces the duration of virus excretion in children hospitalized with rotavirus gastroenteritis (Brussow et al 1987) and an early recovery and 29% reduction in stool volume compared to nontreated children (Mitra et al 1995). Similar success with shortening of duration of diarrhoea with use oh HBC has also been reported (Guarino et al 1994) . No side-effects could be observed in any of those studies and thus suggests a safe therapeutic alternative in the treatment of infectious diarrhoea.

The mechanism of action of HBC in protecting and curing the patients with bacterial and viral pathogens is not yet completely known. However, neutralization of viral and bacterial antigens by the specific antibodies in HBC may be an important factor in the mechanism of early clearance of the pathogens. Binding of an intact immunoglobulin to the antigen (e.g. bacterial consituent) may reduce contact with the gut mucosa and facilitate the elimination of excess of potentially pathogenic substance of alimentary, bacterial or viral origin (Williams 1972).

Clinical studies with a specially prepared concentrated cow colostral antibody (HBC) against rotavirus

and ETEC to evaluate its efficacy in children with diarrhoea due to those pathogens are presently going on at ICDDR, B and will soon be started in Stockholm, Sweden

As the use of hyperimmune bovine colostrum has been encouraging in prophylactic and therapeutic studies it would be interesting to study the effect of immunoglobulin from other animal sources such as chickens, which are easy available in most countries in the world and therefore would be cost-effective.

Antibodies in chickens

Antibodies in chickens (IgY) are normally transferred from serum of the hen to the egg yolk and may reach higher concentrations than in serum (Rose et al 1974). This transfer includes antibodies which have arisen as a consequence of deliberate immunization (Polson et al 1980, Jensenius at al 1981, Hassl et al 987). The amount of antibodies present in a single egg may be more than sufficient as a daily prophylactic dose against gastrointestinal infections, provided that the hen is immunized against the offending microorganism. The neutralizing titre in hens appear to be similar to bovine antibodies (Bogstedt at al 1996, in press).

Chicken antibodies are most probably less stable with regard to proteolysis *in vitro* than bovine antibodies (Shimizu et al 1992), but *in vivo* stability of chicken antibodies (IgY) is still not known. Nor has their prophylactic or therapeutic effect been investigated to date but there is reason to believe, based on animal studies (37-56), that chicken antibodies may also be useful in human therapy. A clinical effect of orally administered antibodies may depend upon blocking of adherence to mucosal surfaces or neutralization of toxins.

The effect might also result from interaction between antibodies and human complement (which leaks out onto mucosal surfaces) or human granulocytes (opsonization of the microorganisms which leads to phagocytosis).

Previous experiments suggest that chicken antibodies do not fix human complement. There are experiments however which the antibodies could be shown to interact with human granulocyte in promoting phagocytosis (Janson et al 1994). The latter finding was unexpected in view of previously published data, but receives support from more recent investigations where a small fraction of chicken antibodies (due to differences in their glycosylation pattern) appears to function as opsonizing antibodies. Antibodies from immunized chickens also, as expected, agglutinate bacteria such as Shigella (Janson et al 1994) and *Campylobacter*. However no studies have so far been made with chicken antibodies on diarrhoea in man. Chicken antibodies against *S. mutans* have recently been shown to exhibit a therapeutic effect in humans (Michalek, S. et al 1996).

Safety of chiken egg yolk immunoglobulin in humans

Chiken egg yolk as nutrients is common to human population all over the world. The Immunoglobulin derived from egg yolk (IgY) can thus safely be used for therapy until there is history of known egg protein allergy. Ig Y specific to *Streptococcus mutans* (organism associated with dental caries formation) has been effectively and safely used in passive immunization to prevent dental caries in humans (Michalek et al 1996). In comparison to the duck egg the probability of containing *S. typhi* in the egg yolk immunoglobulin is extremely low.

Infantile diarrhoeá due rotavirus

Acute infectious diarrhoea is an important health problem and a major cause of morbidity and mortality among young children throughout the world (Guerrant RL, 1990, Levine et al 1986). It also contributes

significantly to the development of malnutrition in children. Enterotoxigenic *E. coli* and rotavirus are leading enteropathogens responsible for diarrhoea in children 6-24 months of age in Bangladesh (Stoll et al 1983, Haque et al 1994). Rotavirus together with ETEC have been associated with up to 45% of all episodes of acute gastroenteritis requiring hospitalization among young children. It has been shown that from 1987 to 1995 serotype G 1-4 have predominated in Bangladesh (Bingnan et al 1995, Unicomb et al 1993, Unicomb- personal communication). The organisms are also considered as predominant enteropathogens in Brazil, (Linhares et al 1989, Guerrant et al 1983) and Mexico (Cravioto et al 1988). The mechanisms responsible for rotavirus-diarrhoea are related to nonspecific histopathological abnormalities of the small intestine. Following infection, enterocytes lining the apical villi are destroyed, and immature crypt enterocytes migrate to the villous tips at an accelerated rate. Diarrhoea in rotavirus infection is associated with a delay in normal differentiation of villous enterocytes, resulting in a mucosa with an inadequately developed transport system (Davidson et al 1977, Kerzner et al 1977) leading to failure of nutrient absorption and osmotic diarrhoea.

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 minimize nutritional consequences.

ORT does not reduce the severity and duration of diarrhoea. This limitation is of practical concern to both the patients and caretakers in diarrhoea management because one of the objectives of the management is to reduce severity of illness in terms of stool volume and duration of diarrhoea. Therefore, demand for useless and often harmful drugs e.g. anti motility drugs as a measure to reduce stool volume and duration of diarrhoea due to these pathogens, is very high. Results of clinical trials with various anti diarrhoea drugs have thus far been disappointing.

In this study we propose to investigate the therapeutic effectiveness of chicken egg yolk immunoglobulin from hens immunized with rotavirus in a double blind randomized clinical trial in children with diarrhoea due to this pathogen.

Specific aim: The aim of the project is:

> To evaluate whether chicken egg yolk immunoglobulin (IgY) with a high titre of antibody against the four common serotype of human rotavirus, reduces the severity and duration of diarrhoea in infants and children aged 4 to 24 months

Rationale

- 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 anti diarrhoeal drugs have been disappointing; it is therefore important to evaluate new approaches to antidiarrheal therapy;
- Antibodies from other species, e.g. hyperimmune bovine colostrum have been used with positive effect both as prophylaxis and therapy.
- The IgY may be more cost effective than HBC in the treatment of diarrhoea due to rotavirus.
- Use of chicken egg yolk immunoglobulin is an immunological approach to treat infections with freeze-dried egg yolk containing a very high titre of antibodies against pathogens like rotavirus. In Japan, these egg yolk immunoglobulin against *S. mutans* is already in the market and used for prevention of dental caries.

- The chicken egg yolk is a highly nutritious food and should generally be regarded as safe with no side effects, unless there is a known egg allergy.

Controlled clinical trial with IgY in infants and small children with rotavirus diarrhoea:

Methods and Procedures

Production of IgY

The study would require around 1, 8 - 2, 0 kilograms of anti - rotavirus antibodies. The hens will be immunized intramuscularly with the four serotype of rota virus antigen namely: WA, RV5, RV3, and ST3. The chicken egg yolk immunoglobulin (IgY) will be given to patients as a freeze-dried powder. The IgY will be produced by Taiyo Kagaku company in Japan and shipped to ICDDR, B Dhaka. The neutralization titre of this immunoglobulin against rotavirus appears to be similar to hyperimmune bovine colostrum (neutralization titre 1:6,000 for a 10% solution) (Bogstedt et al 1996, in press).

Procedures

Infants and children of 6-24 months of age presenting at the outpatient ward of the International Center for Diarrhoeal Diseases Research, Bangladesh (ICDDR,B) with a history of watery diarrhoea of 2 days or less and 4 or more liquid stools during previous 24 hours will be evaluated and if they fulfill the study inclusion and exclusion criteria will be admitted to the study ward. A rapid test for rotavirus (latex agglutination) antigen in stool will be used to ascertain its presence within four hours of admission. The stool samples will also be send for dark field microscopy examination to detect *V. cholerae* and microscopic examination. The children whose stool demonstrates positive latex agglutination for rotavirus, no inavsive picture in stool microscopy and negative dark-field test for *V. cholerae* will be included in the study. The stool will also later be analyzed for the common pathogens (*V. cholerae, Campylobacter, Salmonella, Shigella,* ETEC).

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 breast feeding of those still breast fed during rehydration and maintenance therapy, and **c**) formula milk for non breast fed infants starting after initial rehydration period and **d**) semisolid and solid food appropriate for age during maintenance fluid therapy. Intake of food will be supervised by a health worker and the amount will be recorded in a precoded form. The frequency of breast feeding will also be recorded.

A double-blind randomized placebo-controlled clinical trial will be done. Patients in the treatment group will receive IgY from immunized hens. Patients in the control group will be similarly treated; instead of IgY , they will receive the same amount of IgY from hens not specifically immunized with antigen.

10 g of IgY or non-immunized IgY (according to randomization - as mentioned below) will be dissolved in 10 ml of water at ambient temperature that has been previously boiled and will be fed to the children 6 hourly, starting soon after the patient has been assigned to the study, for 4 days. The daily dose has been calculated from earlier studies with HBC.

The children will be placed on a cholera cot. A paediatric urine collection bag will be used to collect stool and urine separately. The stool will be replaced by ORS as per volume for stool-weight basis. The intake of ORS will be supervised by a nurse or a health worker and will be recorded.

Stool will be tested for rotavirus antigen (immediately by rapid method and later by ELISA) and cultured for detection of toxigenic *E. coli, V. cholerae, C. jejuni, Salmonella and shigella* upon enrollment in the study. Daily stool samples will also be obtained every morning for 4 days and will be processed for

detection of rotavirus by ELISA. Blood for hematocrit, complete count, total solids and electrolytes will be taken on admission only if clinically indicated and will be repeated if required. Intake and output measurements will be instituted using urine bags. Patients will be evaluated every 8 hours and output measurements will be summarized in a precoded form. Patients will be kept in the hospital until cassation of diarrhoea. Body weight and physical examination findings will be recorded daily and time of cessation of diarrhoea will be noted. Rotavirus positive samples will be retained (-20°) for serotyping by ELISA.

We will also send 3-4 random sample of chiken egg yolk immunoglobulin for culture for *S. typhi* before commencement of the study or during the project period.

Randomization

The patients in the study group will receive chicken egg yolk immunoglobulin (IgY) from hens immunized with rotavirus. Patients in the control group will receive same amount of non - immunized IgY. The IgY preparation will be dispensed in identical containers. Two master randomization charts will be prepared by an appropriately trained person who is not connected to the study using random permuted block (to equalize the number of patients in the two groups after short intervals). Bottles containing IgY will be arranged in a sequence that corresponds to the randomization chart and serially numbered. The serial number of the bottles will correspond to the serial number of patients enrolled in the study.

Exclusion criteria

Children with following criteria will **not** be included.

a. systemic infection requiring prompt antibiotic treatment;
b. children with severe degree of malnutrition (marasmus or kwashiorkor)
c. history of bloody diarrhoea.
d. if antibiotic or drugs are prescribed for diarrhoea before coming to the hospital.
e. history of egg allergy

Outcome Variables

Major:

a. Total stool output in g/kg of body weight, from the time of initiation of treatment with IgY until the end of diarrhoea;
b. Duration of diarrhoea (hours) after randomization;
c. Clearance of rotavirus from stool.

Minor:

d. Intake of ORS solution from start of IgY treatment until diarrhoea stops;
e. Vomiting - number and duration during the treatment with IgY.

Sample Size

The present study is largely exploratory (i.e. IgY 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 we calculated the sample size on the basis of duration of diarrhoea. In a group of infants with rotavirus diarrhoea (Sack et al, 1978) the mean duration of diarrhoea after admission to the hospital was 57 hours (SD +/- 26). We expect at least a 30% reduction in duration of diarrhoea with this treatment. To detect a difference of this magnitude at 5% level and 80% power, the sample size is 37 in each group i.e. 74 in both groups. We speculate that a, 20-25% of the children will have more than one pathogen or rotavirus beyond serotype 1-4 in their stool and thus will be dropped from

the final analysis. Considering this fact, the sample size will be 82. With a further 10% drop out because of study withdrawal and non-compliance, the total sample size will be 90 children.

Informed Consent

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

Definitions

1. Duration of diarrhoea: the time in hours from initiation of the study treatment until end of diarrhoea.
2. End of diarrhoea: passage of the last liquid or semi liquid stool prior to two formed/soft stools for 16 hours.
3. 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).

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 randomized patients will be included in the analysis of the study. Data from patients withdrawn will be included up to the time of withdrawal. A supplemental analysis in which such patients are excluded will also be made.

Data Analysis

The study groups will be compared for all the variables prior to intervention (i.e. initiation of IgY). 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' tests on primary data or, after appropriate transformation when indicated and also by an equivalent non parametric test e.g. Mann Whitney U test, Dichotomous outcome measures will be compared by Chi square or Fisher's exact test as appropriate.

Study Schedule.

1. Procurement of supplies and standardization of methods and development/ testing of data forms and recruitment and training of personnel 1 - 2 months.
2. Conduct of the trial 12 - 13 months
3. Analysis of data and preparing report 2 - 3 months

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BUDGET PROPOSAL

Project title: "Evaluation of Hyperimmune Egg Yolk Globulin (HEYG) in children with per acute diarrhoea"
Project Duration: 1 year 6 months from starting

Name of P.I : Dr. S.A. Sarker

Line item	1st year	2nd year 6 month	Total
Personnel local salaries			
Dr. S. A. Sarker (10%)	1,440	770	2,210
Dr. N.H. Alam (05%)	670	358	1,028
Dr. P.K. Bardhan, (5%)	737	394	1,131
Research Physician x 1 (100%)	2,000	1,070	3,070
Female Volunteer-3 (100% time)	1,500	803	2,303
Budget and Cost Officer-05% time	228	122	350
Sub-total	6,575	3,517	10,092
LOCAL TRAVEL: (General transport & conveyanc - Travel -Perdiem	250	250	500
INTERNATIONAL TRAVEL: (Ticket, transport etc) -Travel -Perdiem		2,500	2,500
Sub-total	0	2,500	2,500
SUPPLIES & MATERIALS			
-Drug/medical supplies	200	100	300
-Laboratory Supplies	200	100	300
-Others	200	100	300
	600	300	900
OTHER CONTRACTUALS			
-Repair, maintenance, renovation etc.	200	100	300
-Service charge; daily wager, short term emp)	200	100	300
Sub-total	400	200	600
INTER DEPARTMENTAL SERVICES			0
-Medical Illustration	50	50	100
-Xerox, Library Service	100	100	200
-Lab and Pathological test	1,500	1,500	3,000
-Patient hospitalization (90x6dayx$30)	8,000	7,800	15,800
Sub-total	9,650	9,450	19,100
Total Operating Cost	17,475	16,218	33,693
Indirect Cost 7% on Operating Cost	1,223	1,135	2,358
Total Project Cost	18,698	17,353	36,051

5/5/96

CONSENT FORM

(will be read and explained clearly before consent is obtained)

Title: **Evaluation of Chicken Egg Yolk Immunoglobulin (IgY) in the treatment of diarrhoea due to rotavirus in children**

Your child is suffering from acute watery diarrhoea. The major treatment of this disease is oral rehydration therapy to correct dehydration and to replace the continued stool loss. But the oral rehydration therapy does not reduce the volume of stool and duration of diarrhoea. It is therefore important to find out a new approach to reduce the stool loss and duration of diarrhoea. It is expected that treatment with Chicken Egg Yolk Immunoglobulin (IgY) will reduce the severity of diarrhoea. International Center for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) is carrying out a study to evaluate the efficacy of Chicken Egg Yolk Immunoglobulin (IgY) in the treatment of diarrhoea. We request you to allow your child to participate in this study. If you agree, the following procedures will be followed.

1. Your child will be kept in the hospital until diarrhoea stops and will be provided routine diarrhoea care.

2. During this period, your child will receive any one of the two cow colostrum for four days. They are: (i) Immunized chicken Egg Yolk Immunoglobulin, (ii) Egg Yolk Immunoglobulin.

3. Stool will be taken daily for examination for 3 days.

4. At any stage of the study, you may withdraw your child from the study. In this case, the child will be given the standard treatment of the hospital.

The study will not cause any harm to the child.

If you agree to participate in this study, please give your signature below.

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Signature of the Investigator	Signature of witness	Signature or thumb impression of legal guardian

সম্মতি পত্র

(সম্মতি দেয়ার পূর্বে এ পত্রটি ভালভাবে ব্যাখ্যা করা হবে)

গবেষনার নামঃ **শিশুদের রোটাভাইরাস, জনিত ডায়রিয়া রোগের চিকিৎসায় উচ্চ প্রতিরোধ ক্ষমতা সম্পন ডিমের কুসুমের কার্যকারীতা পরীক্ষা।**

আপনার শিশু পাতলা পায়খানায় ভুগছে। খাবার স্যালাইনের মাধ্যমে এবং শরীরের পানি ও লবণের ঘাটতি পূরণ করাই হচ্ছে এ রোগের অন্যতম চিকিৎসা। কিন্তু খাবার স্যালাইন ডায়রিয়ার মেয়াদ ও পায়খানার পরিমান কমাতে সাহায্য করে না। ডায়রিয়ার মেয়াদ ও পায়খানার পরিমান কমানোর জন্য নতুন উপায় বের করা তাই অত্যন্ত জরুরী। আমরা আশা করি ডিমের কুসুম থেকে তৈরী অধিক প্রতিরোধ ক্ষমতা সম্পন্ন ইম্মিউনোগ্লোবিউলিন ডায়রিয়ার ভয়াবহতা কমাবে। আন্তর্জাতিক উদরাময় গবেষণা কেন্দ্র এ রকম একটা গবেষণা চালাচ্ছে। আপনার শিশুকে এ গবেষণায় অংশ গ্রহণে অনুমতির জন্য আপনাকে অনুরোধ করছি। আপনি রাজী থাকলে আমরা নিম্নের বর্ণিত কার্যক্রম অনুসরন করবো।

১। আপনার শিশুকে ডায়রিয়া থেকে আরোগ্য না হওয়া পর্যন্ত এ হাসপাতালে রাখা হবে এবং প্রয়োজনীয় চিকিৎসা প্রদান করা হবে।

২। এ সময় আপনার শিশুকে ডিমের কুসুমের ইম্মিউনোগ্লোবিউলিন এর যে কোন একটি ৪ দিন ধরে খাওয়ানো হবেঃ

(১) ডিমের অধিক কার্যক্ষম সম্পন্ন ইম্মিউনোগ্লোবিউলিন
(২) শুধুমাত্র ডিমের ইম্মিউনোগ্লোবিউলিন

৩। পায়খানা জীবাণু নির্ণয়ের জন্য প্রথম তিন দিন মল পরীক্ষা করা হবে।

৪। গবেষণা চলাকালীন যে কোন সময়ে আপনি আপনার শিশুকে গবেষণা থেকে প্রত্যাহার করে নিতে পারবেন। সেক্ষেত্রে হাসপাতালের নিয়মিত চিকিৎসার কোন ত্রুটি হবেনা।

এ গবেষণায় শিশুর বিপদের কোন আশংকা নেই। যদি এ গবেষণায় রাজী থাকেন তবে দয়া করে নীচে স্বাক্ষর করেন।

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গবেষকের স্বাক্ষর	সাক্ষীর স্বাক্ষর	অভিভাবকের স্বাক্ষর/বামহাতের বৃদ্ধাংগুলির ছাপ
তারিখঃ----------	তারিখঃ----------	তারিখঃ----------