Attachment 1.
(FACE SHEET)

Date 28/6/92

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator Dr P.K. Bardhan
Application No. 92-020
Title of Study "Helicobacter Pylori Infection as a ~~Risk Factor for Acute~~ Diarrhoea and Persistent Diarrhoea: a Prospective Case-Control Study"

Trainee Investigator (if any) 38
Supporting Agency (if Non-ICDDR,B)

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.

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

Trainee

A-031962

Hypothesized

RESEARCH PROTOCOL

Title: Helicobacter Pylori Infection as a Risk Factor for Acute Diarrhoea and Persistent Diarrhoea: a Prospective Case-Control study

Principal Investigator : Dr. P.K. Bardhan

Co-Principal Investigator : Dr. D. Mahalanabis

Co-Investigators : Dr. S. A. Sarkar
Dr. A. S. G. Faruque
Dr. F. Qadri
Dr. John Albert

Collaborating Investigator(s): Prof. Klaus Gyr
Dr. Beat Meyer
Dr. Chris Beglinger
University of Basle, Switzerland

Consultant : Dr. R.B. Sack

grant ?

Amount : US $ 96,970

Starting date : As soon as possible

Ending date : 2 years after start

H. pylori
Persistent diarrhoea
genetic factor

Approved by: Associate Director
Clinical Sciences Division

Date: 28/6/92

Abstract

Hypochlorhydria is known to be a predisposing factor to repeated gastro-intestinal infections. *Helicobacter pylori* is now accepted as a major cause of human gastritis, and a striking association has been noted between presence of H. pylori and gastric hypochlorhydria in humans. Recent evidence suggests that H. pylori is also associated with persistent diarrhoea in children, and may be a risk factor for infectious diarrhoea. The present study proposes to ascertain if H. pylori infection is a risk factor for acute and persistent diarrhoeas in children in a prospective case- control design. The cases will consist of 100 children (3-35 months) with acute diarrhoea and 100 children (3-35 months) with persistent diarrhoea. The controls will consist of 200 neighbourhood children (who would be attending ICDDR,B in case they develop diarrhoea) and 200 randomly selected children. The controls will be selected by age-matching into two broad age strata : 3-18 months and 19-35 months, and the recruitment will be concurrent. The disease status will be ascertained by history and observation, whereas the exposure status will be determined by ^{13}C-urea breath test and serology. Data about other probable confounding variables such as socio-economic status, nutritional status, feeding, sanitation, cell mediated immunity, faecal pathogens, vitamin A and zinc status will also be collected. Hopefully, this study will be able to identify any association between H. pylori infection and diarrhoea in children.

GENERAL OBJECTIVE

To examine if *Helicobacter pylori* infection is a risk factor in acute and persistent diarrhoeas in children.

BACKGROUND:

The following points are considered for justification of such a study :

1. Evidence that *H. pylori* infection occurs in the age group susceptible to acute and persistent diarrhoea in developing countries, for example studies in Lima, Peru (1) and in The Gambia (2);

2. Evidence that *H. pylori* is asociated with persistent diarrhoea as shown in the Gambian study (2).

3. Recent evidence that pathogens isolated during the acute phase of diarrhoeal illness in children are as a rule different from those isolated from the same children when the diarrhoea persisted beyond 14 days indicating a host factor or other factors (Dr. Abdullah Baqui's community based study in rural Bangladesh, PhD Thesis, Johns Hopkins University).

4. Some indication that *H. pylori* infection leads to gastritis and may cause hypochlorhydria which predisposes one to G.I. infection.

H. pylori infection in children :

Helicobacter pylori is now accepted as the major cause of chronic gastritis in humans (3). Factors known to be associated with gastritis include low socio-economic class, large family size, crowding etc. *H. pylori* infection may be acquired at any age but once the infection is acquired, the duration appears to be long. Age-specific prevalence of H.pylori infections (mainly sero-epidemiology) is higher in developing countries than developed countries. Within a specific country, age-specific prevalence is higher in the low socio-economic group whether assessed by income, housing, educational level or others. Sanitation and hygiene appears to be important factors associated with acquisition of *H.pylori infection* (1). The mode of transmission is unknown but the geographic and social patterns of *H. pylori* infection are consistent with faecal-oral transmission as one major pathway (3). Recent data from Lima, Peru (1) demonstrated a direct association between the prevalence of *H. pylori* infection and source of drinking water. Furthermore, recently it was shown that motile spiral forms of *H. pylori* can survive for at least a week in river water (4). *H. pylori* has not yet been isolated from the environment but the technology is now available (e.g. PCR techniques can address this question). In developed countries *H. pylori* infection is infrequent in children.

In developing countries some recent studies indicate that *H. pylori* infection is acquired in infancy and in early childhood in. In Lima, Peru as high as 25-50% of the children under 2 years were positive for *H. pylori* (1). In Thailand 74% of 1-4 year old children in an orphanage were sero-positive for *H. pylori* (5). In India 60% of 3-10 year old children were found to be positive for *H. pylori* (6). In The Gambia (2) in the age group under 30

months 12% of well nourished children (n=70), 28% of marasmic children (weight-for-height less than 75%, n=49), and 53% of severely malnourished children with chronic diarrhoea were sero-positive for *H. pylori*.

H. pylori infection and chronic diarrhoea in children:

The results of the study in The Gambia (2) indicate, for the first time, a close association between *H. pylori* infection and chronic diarrhoea with malnutrition as well as with severe malnutrition without diarrhoea. The association however was not adjusted for confounding variables. In this study 12% of 70 well nourished children, 28% of marasmic children (weight-for-height less than 75% NCHS), and 53% of malnourished children with persistent diarrhoea were positive for *H. pylori* infection. The odds ratio for having *H. pylori* infection was 14 times higher in children with severe malnutrition and chronic diarrhoea and 3 times higher in children with severe malnutrition without diarrhoea (our calculation of the Gambian data). In this population 15% of children aged 0-19 months, 27% of children 20-39 months, and 46% of children 40-60 months were sero-positive for *H. pylori*.

Pathogens in acute vs. persistent diarrhoea :

In a recent study Dr. Baqui has provided evidence that pathogens isolated during the acute phase of diarrhoeal illness in children are as a rule different from those isolated from the same children when the diarrhoea persists beyond 14 days indicating host factors or other factors responsible for the persistence of diarrhoea (Ph.D thesis, Johns Hopkins University - A-H Baqui).

***H. pylori* and gastric acid secretion:**

There is evidence that *H. pylori* infection leads to gastritis and may cause hypochlorhydria which is known to predispose a child to repeated gastrointestinal infection and probably persistent diarrhoea. Hunt and his colleagues recently reported the effect of *H. pylori* on acid production, using ^{14}C-aminopyrin uptake by isolated guinea pig parietel cells. *H. pylori* caused a reduction of about 80% in basal acid secretion, and histamine-stimulated acid secretion was reduced to 50% within 15 minutes of inoculation with H. pylori (7). There are also reports describing *H. pylori*-associated gastric hypochlorhydria in man (8,9,10). Although a striking association between the presence of *H. pylori* and hypochlorhydria exists the question of cause and effect is still obscure. In 1983 Marshall, rendered himself temporarily hypochlorhydric with cimetidine and then swallowed cultures of *H. pylori*, obtained from a patient with gastroenteritis! Although gastritis and *H. pylori* were detected in his follow-up tissue specimens (11,12), a definite association of the organism with hypochlorhydria could not be established, as gastric acid secretion before and following challenge was not monitored in that experiment. In developing countries it appears that *H. pylori* infection occurs in early life; furthermore, a high prevalence of hypochlorhydria is known to exist in poor countries. A clear association between the two has not yet been established.

Because of poor environmental conditions in developing countries, people are very often exposed to enteric pathogens such as *V. cholerae*, *Shigellae* and *E. coli*. Low gastric acid production has been found to be associated with a

high risk of cholera and E.coli diarrhoea in those communities (13,14). It is not unlikely that *H. pylori*-associated hypochlorhydria leads to increased susceptibility to enteric pathogens.

Urea breath tests and serology for *H. pylori* infection (15):

Urea breath tests were first used in the 1950's when study of gastric urease was fashionable. However, when it was known that urease was not of mammalian origin there was not much interest in this test until recently when it was adapted to diagnose urease-producing *H. pylori* infection.

The principle of the urea breath test is that, in the presence of the enzyme urease, orally administered urea is hydrolysed to CO_2 and ammonia. If the urea carbon is labelled with either stable isotope ^{13}C- or radioactive ^{14}C- it can be detected in the breath as labelled CO_2. *H. pylori* is the most common urease-containing gastric pathogen and therefore a positive urea breath test can generally be equated with the presence of *H. pylori* infection. Urea breath test for *H. pylori* has a high degree of sensitivity and specificity. It should be pointed out that serologic tests to detect antibodies against *H. pylori* are also available and, because the infection is of very long duration, a positive serologic test is generally sufficient to diagnose an active infection. However, if *H. pylori* infection is eradicated or suppressed by treatment serologic tests are still likely to remain positive. Finally, ^{13}C- urea breath test is a simple, inexpensive, non-invasive, safe and accurate method of diagnosing active *H. pylori* infection (see Annex 2).

Hypotheses:

1. *H. pylori* infection in a small child causes gastritis and hypochlorhydria which persists for some time;

2. Such a child becomes more susceptible to infection with bacterial diarrhoea pathogens; she/he may also be more susceptible to colonisation of upper small bowel with faecal coliforms;

3. Such a child becomes more susceptible to acute diarrhoeal illness;

4. Once acute diarrhoea occurs in such a child with gastritis and hypochlorhydriait lasts longer than usual in a child than a child without H.pylori infection for the following reasons:

   a. due to increased susceptibility to enteric pathogens

   b. due to increased susceptibility to colonization of small bowel with faecal coliforms

SPECIFIC OBJECTIVE

1. Does H.pylori infection increase a child's susceptibility to acute diarrhoea and/or persistent diarrhoea ?

2. If so, is this associated with age and thus indirectly indicates the role of recent infection with H.pylori ?

3. How common is H.pylori infection in this child population (controls)?

STUDY DESIGN

A clinic-based case-control study with community controls will be used. A case-control design is proposed for cost reasons and for ethical reasons because in a cohort study, once we find a child with diarrhoea, it will be difficult to justify not to treat the condition and follow the child in order to simply observe the consequence of such an infection.

Selection of cases and controls:

Before defining the cases and controls we provide in Annex 1 some relevant general comments largely from epidemiology texts (Rothman KJ - Modern Epidemiology, 1986) to clarify the basis of defining cases and controls for this case-control study.

Definition of cases and controls:

Cases -

Group 1. Acute diarrhoea: Children aged 3 months to 35 months with acute watery diarrhoea of 3 day or less attending ICDDR,B treatment centre will be considered for inclusion in this group. A random sample of those who stayed long enough at the treatment centre or as an inpatient and in whom presence of diarrhoea has been confirmed by observing the passage of at least one liquid stool during hospital stay will be included as a case in this group. They will be followed up at home to assure that diarrhoea does not last longer than 10 days from onset of illness. Those having diarrhoea longer than 10 days will not be included in this group. Therefore, the group will consist of cases of unequivocal acute diarrhoea of "short duration".

Group 2. Persistent diarrhoea: Children aged 3-month to 35-month old attending ICDDR,B treatment centre with a history of acute diarrhoea of 10 days or more but less than 4 weeks will be considered for inclusion in this group. Any intervening symptom-free period of more than 48 hours will be regarded as interruption of illness. A random sample of those observed for sufficient duration to ascertain that they still have diarhoea (i.e. passage of a liquid stool) will be included. Patients who have a history of less than 14 days of diarrhoea will be followed up to ascertain diarrhoea duration and if the total duration is less than 14 days then they will not be included in this group. Therefore, the group will consist of cases of unequivocal persistent diarrhoea as conventionally defined as diarrhoea that starts acutely but persist beyond 2 weeks.

Exclusion criteria for cases :

1. Dysentery: history of blood with or without mucus in stool
2. Presence of any significant systemic illness (e.g. pneumonia, meningitis)
3. Children accompanied by a person not generally responsible for the child

Controls

1. Control Group 1 : Children from the neighbourhood of the cases will be selected as controls. For each case in group 1 and in group 2, a control will be selected. Controls will be selected by two age strata (i.e. 3months to 17 months, 18 months to 35 months) according to the age of the case.

To assure that the control is likely to be brought to ICDDR,B if he/she suffers from similar illness, parents of the case will be asked about other families in the neighbourhood who also go to ICDDR,B. Controls will be taken from such families. If such families with a child fulfilling the selection criteria are not available then other families in the neighbourhood will be evaluated for their familiarity and use of ICDDR,B hospital. A child in the same age stratum without any history of diarrhoea in the preceding 2 months will be selected as a control. A follow-up visit will be made 2 weeks later to ascertain that the child has had no diarrhoea following inclusion as a control. A control will be enrolled within 2 weeks of enrolling the corresponding case i.e. enrollment will be concurrent.

2. Control Group 2 : For each case in group 1 and in group 2, a random community control will be selected, similarly by two age strata (i.e. 3m to 17 month, 18 month to 35 month) according to the age of the case.

After selecting a control group 1 subject, a random control will be sought for by moving approx 100 metres on right or left direction (decided by a toss of coin) and then searching for a subject fulfilling the criteria - a child in the same age stratum without any history of diarrhoea in the preceding 2 months. If consent to participate is not given, then the name and address will be noted alongwith the cause of refusal, and the search will continue on the same direction till a control is enrolled. A follow-up visit will be made 2 weeks later to ascertain that the child has had no diarrhoea following inclusion as a control. Enrollment of group 2 controls will also be concurrent.

(For discussion on criteria for cases and controls see annex 1).

Other risk factors and confounding variables :

1. Protein energy malnutrition:

Protein-energy malnutrition is known to be associated with hypochlorhydria and this factor will be adjusted for in the analysis; anthropometric indicators of protein-energy malnutrition will be used. A caveat is in order here, if hypochlorhydria in the malnourished is mediated through H.pylori infection then this adjustment will lead to an underestimation of the exposure/disease relationship;

2. Acquired, albeit temporary, immunodeficiency due to recent infection with viruses as for example measles and other infections. This will be adjusted for by using skin tests with multiple antigens for delayed type hypersensitivity reaction as an indicator of cell-mediated immunity;

3. Micro-nutrient deficiency:

Vitamin A deficiency and zinc deficiency could be confounding variables; clinical signs and symptoms of vitamin A deficiency will be carefully recorded; appropriate sample of urine will be taken for analysis of zinc;

4. *Age and sex:* They will be adjusted in the analysis phase;

5. *Specific enteropathogens:*

Specific enteropathogens known to be associated with acute diarrhoea (Cholerae, salmonellae, Shigellae, rotavirus, ETEC) and particularly with persistent diarrhoea , e.g. (*Cryptosporidia, Giardia lamblia, enteroadherent E. coli*) will be looked for.

Sources of bias:

1. *Misclassification of disease:*

Ascertainment of acute diarrhoea and persistent diarrhoea are unlikely to be misclassified due to the obvious nature of the disease; in addition the patients will be observed to ensure that they still have diarrhoea before they are included in the study as cases; controls are neighbourhood controls with no existing diarrhoea and history of diarrhoea and this again is unlikely to be misclassified;

2. *Ascertainment of exposure status:*

Diagnosis of H.pylori infection using 13C- urea test is highly specific and sensitive and therefore misclassification of exposure status should be very small. However, the gastric acid status will not be directly measured and therefore it will still remain uncertain whether the children with H.pylori infection have low gastric acidity at that point of time. We are aware that gastritis and probably hypochlorhydria following H.pylori infection lasts for some time; yet, it may happen that although H.pylori infection is not cleared, the gastric acidity may have returned to near normal in some of the cases in whom the ^{13}C- urea test is positive however. This may be age-related since the younger the age the more likely that the infection is more recent. This bias will be minimised by adjusting for the age. We will also evaluate the odds ratio according to age strata and look for a trend.

Sample size calculation:

In Bangladeshi adults undergoing gastroscopy, more than 75% were found to harbour H. pylori as determined by examining gastric biopsy (Bardhan, Azad, Islam et al - ICDDR,B unpublished data). Recent reports from The Gambia showed that among malnourished children with persistent diarrhoea 53% were seropositive for H.pylori. In rural under-five children in The Gambia 15% were seropositive in the age group 0-19 months and 27% were seropositive in 20-39 month old children. In Lima, Peru (1)an estimated 25% infants under one year were seropositive in the area with protected drinking water while about 50% of under 1 year old children were seropositive in the area supplied by less protected water. In the absence of information among Bangladeshi children we would calculate sample size assuming a 20% exposure rate, 80% power and at

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5% significance level to detect an odds ratio of 2.5 (the odds ratio for persistent diarrhoea in Gambian children was calculated to be about 3.4). The number of children in each group is then 128 (including 25% allowing for confounding variables) in each group. This means recruitment of 128 acute diarrhoea cases and 128 persistent diarrhoea cases; the number of controls by this study design will be 256. Using 25% exposure rate for H.pylori (as in Peru) the sample size would be a good deal smaller.

If we use twice the number of controls as cases (i.e. use all the controls in this study) then the number of cases would be reduced to 96 for each type of cases.

Assuming a more conservative exposure rate of 15%. The number of cases of each type of case will be about 166. With controls 2 times the number of cases the sample size for cases would be about 125 for each type of cases. In view of the high cost of ^{13}C-urea test we propose to use the sample size calculation based on an expected exposure rate of 20%. Therefore our sample size would be about 100 cases of acute diarrhoea, 100 cases of persistent diarrhoea and 200 controls.

Collection of data:

After enrollment into the study a detailed clinical history will be recorded and a thorough physical examination performed by a physician. Examination will include nutritional anthropometry (height/length, body weight and mid-arm circumference) and a search for vitamin A deficiency signs/symptoms. Stool samples for microscopy (including for cryptosporidium and giardia), culture for ETEC, EPEC, *Campylobacter jejuni, Aeromonas, Plesiomonas shigelloides, Salmonellae, Shigellae, Vibrios* and ELISA for rotavirus will be obtained. *E. coli* strains will be tested for adherence. Each subject will also be interviewed by a trained interviewer who will record details of social and demographic data on a pretested form.

Serology (See Annex 2)

^{13}C- Urea breath test (See Annex 2)

Cell-mediated Immunity:

"Multitest" (Institutet Merieux) will be used to test delayed type hypersensitivity reaction to indicate cell-mediated immunity status. Multitest is an applicator made of acrylic with 8 heads of tines loaded with 7 different antigens and a glycerin control. If the capacity to respond is present, the subject will show a reaction to several of these recall antigens similar to tuberculin "Tine" test. The antigens are tetanus, diphtheria, streptococcus, tuberculin, candida, trichophyton and proteus.

Urine Zinc level :

Urinary zinc level will be measured as an indicator of zinc status. A random sample of urine will be collected and stored in cool-box immediately after collection till brought back to the laboratory, and will be stored at -20°C until assayed. Zinc will be determined by atomic absorption spectrophotometry, and urinary creatinine will be measured by picric acid method. Urinary zinc level will be expressed as zinc in mmols/mol of creatinine (16).

Data Analysis:

We will follow the standard analysis for such case control studies as described by Schesselman (1982). At first a crude analysis will be carried out to evaluate the odds ratio (& 95% confidence interval) between main exposure of interest i.e. H. pylori infection and disease; the controls will be compared with acute diarrhoea and with persistent diarrhoea separately and we will also look for a trend. This 2x2 table will then be stratified by the likely confounding variables one at a time and a summary odds ratio (Mantel-Haenzel) will be calculated; if the Mantel-Haenzel odds ratio deviates more than 10% from the crude odds ratio then it will be included in the subsequent analysis in a logistic regression model. In addition, the confounding variables that are biologically plausible confounders but did not qualify by the above criteria will also be considered for inclusion in the model. Logistic regression model will be structured. First a basic model will be fitted between the exposure of primary intent and disease state; other risk factors and confounding variables will then be introduced in the model. Closely associated covariates will be entered one at a time or a composite indicator will be developed for them if found relevant.

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ANNEX 1

A case-control study may be restricted to any type of case that may be of interest; the cases however must be selected independently of exposure, but the prevalence of exposure among cases may nevertheless be higher or lower than the prevalence among other persons with disease if the cases differ by factors such as age, sex or severity from other persons with disease. IN a case control study, it is wrong to advice that cases should be representative of all persons with the disease and that controls should be representative of the entire non-diseased population. Just as follow-up studies can be based on special cohorts rather than on general population, it follows that case-control studies can be conducted by sampling cases and controls from within such special cohorts.

The objective in selecting controls is to choose individuals representative of those who, had they developed disease, would have been considered for selection as cases and of course, to choose these controls independently of exposure. It follows that cases identified even in a single clinic or an appropriate sample of them are possible case series as long as it is understood that the population from which the controls derive is restricted to those people who would have been considered among cases had they developed the disease.

The above discussion would raise the question of generalization. The process of generalisation beyond a set of observations requires a judgement about the features of observations that may be extrapolated. Such judgements require an understanding of which conditions are relevant to generalisation. Some contend that generalisation from a study group depends on the study group being a representative subgroup of the target population, in the sense of a sample whereas others (Reichenbach, 1951) considers scientific generalisation as an art. If scientific generalisation were simply a matter of statistical generalisation it would be limited literally to those individuals who might have been included, through sampling, as study subjects. If this "misconception" was valid, there would be no application to humans of any results obtained from animal research. In addition, every population would require its own set of epidemilogic studies, and these studies would have to be repeated for every new generation. With our knowledge that H.pylori infection causes gastritis and hypochlorhydria and consequent susceptibility to enteric infection of the upper small bowel we are in a position to use our judgement on generalisation. To consider an epidemiologic example, from a study of smoking and lung cancer in men, one might generalise the results to a target population of women; presumption is that being male is irrelevant to the carcinogenic action that smoking has on lung tissue, a judgement based on knowledge about the mechanism of carcinogenesis and biologic similarity between male and female lungs. Epidemiologic study designs are usually stronger if subject selection is guided by the need to make a valid comparison which may call for severe restriction of admissible msubjects to a narrow range of characteristics, rather than by a futile attempt to make the subjects representative, in a sampling sense, of the potential target populations. Study groups selected for characteristics that enable a study to distinguish effectively between competing scientific hypothesis would enhance the ability to obstruct a mpre general statement from observations than the groups that are representative of larger populations in the statistical sense. The definition of cases and controls have been based on the above discussion.

Annex 2

Serology :

Helicobacter pylori colonisation of the human gastric mucosa stimulates a specific systemic humoral immune response (Rathbone,1986). Although early investigations utilised agglutination and complement fixation methods (Jones,1986), recent studies have demonstrated the suitability of ELISA for detection of H. pylori specific antibodies (Rathbone,1989; Evans, 1989). Specific ELISAs have been found useful as diagnostic tests with both specificity and sensitivity exceeding 90%, and have been extensively utilised for serodiagnosis of H. pylori infection in children from various countries (Glassman,1990; Czinn,1989,1991; Graham,1991; Sullivan,1990; Perez-Perez,1990; Al-Moagel,1990; Holcombe,1992; Klein,1991; Mitchell,1988; Megraud,1989; Drumm,1990; Oderda,1989;1991;). The prevalence of H. pylori specific antibodies in children below 9 years of age varies from country to country even among the developing world - 60% in India, 42% in Saudi Arabia, upto 74% in Thailand, 36% in Papua New Guinea, 48% in Peru, 45% in Algeria, 55% in Ivory Coast, and as high as 82% in northern Nigeria. Though the serological techniques previuosly was largely confined to the research laboratories, availability of commercial ELISA has made it possible to be used in also routine diagnostic laboratories (Crabtree,1991).

Blood samples from acute diarrhoea and persistent diarrhoea patients (2 ml) will be obtained after the patient has been stabilised and within 24 hours of enrollment; sera will be immediately seperated and stored at -20^{o}C until assayed. From mothers of breastfed children seperate permission will be sought for collection of blood and breast milk for determination of specific anti-H. pylori IgA. The serological results from cases will be used for confirmation of H. pylori infection in the cases. No attempt will be made for collection of blood from the neighbourhood controls. This would mean that it will be possible only to compare the serological results between acute diarrhoea cases and persistent diarrhoea cases.

Serum IgG H. pylori antibodies will be measured using a semi-quantitative commercial ELISA kit (Bio-Rad, GAP Test) according to the manufacturers instructions. Essentially, semi-quantitative titres 3+, 2+, 1+, and +/- are respectively assigned depending upon the dilution at which cutoff points are arrived at. This ELISA test utilises purified H. pylori antigen prepared with DEAE-ion exchange chromatography.

Breast milk samples will be collected by manual expression, assissted by a female assisstant if necessary. It will be then centrifuged, the middle layer aspirated, and then frozen at -20^{0}C until assayed. IgA determination will be done by ELISA as described by Perez-Perez et al (1988). Sonicated whole-cell antigen will be prepared from a pool of three H. pylori strains isolated in Bangladesh. The screening serum and breast-milk dilutions will be 1:50, and peroxidase conjugates of goat anti-human IgA will be diluted 1:500. Positivity will be considered when the optical density value will be greater than mean plus 3SD for the results obtained from a panel of reference sera.

^{13}C-Urea breath test :

This test is based upon the principle that in the presence of the enzyme urease in stomach, orally adminstered urea will be hydrolyzed into CO_2 and ammonia. If the urea carbon is labeled with the stable isotope ^{13}C, then it can be detected as labeled CO_2. H. pylori is the commonest urease-producing gastric pathogen, and therefore a positive urea breath can generally be equated with the presence of an H. pylori infection (Graham, 1991). The urea breath test has proven to be very robust, attaining specificity and sensitivity greater than 90% (Dill, 1990). This non-radioactive, non-invasive test have been successfully utilised as a diagnostic tool in H. pylori infection (Graham, 1987; Logan, 1991; Eggers,1989; Klein, 1989; Cooreman, 1990; Lotter, 1990), and was found useful even in children (Klein, 1991).

After obtaining a baseline breath sample at a fasting state (2 hours fast), the test dose of ^{13}C-Urea at the dose of 5 mg/kg body wt. will be adminstered alongwith a liquid meal (for delaying gastric emptying), and then breath samples will be collected every 10 minutes for 1 hour. Breath samples will be collected through a two-way non-rebreathing paediatric mask into vacutainer tubes in duplicate, and will be shipped to Basle, Switzerland, where $^{13}CO_2$ will be estimated by automated gas-isotope ratio mass spectrometry by the collaborating investigators, i.e., Prof. Klaus Gyr and his colleages. An increase in the $^{13}CO_2/^{12}CO_2$ ratio in the breath samples after the test dose of ^{13}C-Urea compared to that of the fasting state will indicate a positive test.

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Tasks of individual investigators

1. Dr. P.K. Bardhan

Design, conduct (primary responsibility), data management, analysis, interpretation of the study results;

2. Dr. D. Mahalanabis

Design, supervision, analysis (primary responsibility) and interpret.

3. Dr. Shafiqul Sarkar

Conduct of the study, data management;

4. Dr. John Albert/Dr. F. Qadri

Conduct/supervise H. Pylori serology, supervise other routine lab tests;

5. Prof. K. Gyr

Supervise 13C-urea test, assist in interpretation of the findings and report writing;

6. Dr. Beat Meyer, Basle, conduct/supervise 13C-urea test

(breath samples), interpret results.

7. Dr. R.B. Sack

Advise on design, interpretation of the findings, supervise lab work at ICDDR,B, give overall guidance.

BUDGET

		1st year	2nd year	Total
Personnel				
Dr. P.K.Bardhan	20%	2,110	2,280	4,390
Dr. S.A.Sarkar	10%	1,020	1,100	2.120
Dr. A.S.G.Faruque	10%	1,100	1,220	2,320
Sr. Health Asstt (2)	100%	8,700	9,150	17,850
Field Worker (3)	100%	4,740	4,740	9,480
Field Asstt (3)	100%	2,850	2,850	5,700
		20,520	21,340	41,860
Laboratory Tests				
Stool M/E and C/S		8,660	7,300	15,960
Urine Zinc		2,100	1,800	3,900
Creatinine		1,450	1,250	2,700
Anti-H.pylori IgG (Serum and Breast milk) Commercial Kits		5,150	4,700	9,850
CMI (Kits)		3,500	3,000	6,500
Breath-Urea Test				
^{13}C-Urea		3,900	3,300	7,200
Breath Analyses		-	-	-
		24,760	21,350	46,110
Supplies (Vacutainer tubes, vials, Micro-centrifuge tubes, Micro-haematocrit capillary tubes, PUC bags, chemicals, syringes, etc.)		2,000	1,500	3,500
International Transport		500	500	1,000
Local Transport		800	700	1,500
Office supplies and Stationaries		500	500	1,000
Data Analysis		500	1,000	1,500
Printing and publication		-	500	500
TOTAL		49,580	47,390	96,970

CONSENT FORM

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

International Centre for Diarrhoeal Disease Research, Bangladesh is planning to undertake a study in Clinical Research Centre (CRC), Dhaka to see whether infection H. Pylori is a risk for developing acute or persistent diarrhoea in children 3-35 months.

We believe this knowledge will bring benefit to the young children. The results may help in designing future action programme for control of diarrhoeal diseases.

We request you to allow your child to participate in this study. If you agree, the following procedures will be followed.

1. A physician will examine your child in general with particular attention to dehydration, nutritional disorders, and other associated health problems. The child will be given appropriate treatment as required If necessary the child will be followed at household till recovery.

2. On admission, information on illness and socioeconomic background will be collected. Anthropometric measurements will be done. A drop of blood from finger tip and stool sample will be collected for laboratory tests. A simple breath test will be performed and two samples of breath will be collected in tubes. A multiple antigen skin test will be applied in the forearms of your child.

3. The study involves no risk. We will maintain the confidentiality of the information given to us. At any stage of the study, you may withdraw your child from study; but his/her routine treatment by us will not be hampered.

4. If you have any question to ask, we shall be happy to answer them.

5. If you agree to participate in this study then please sign below.

---------- ----------

Signature of the Principal Investigator

Signature or left thumb impression of the legal guardian

Date: ----------

Date: ----------

Title: Helicobacter pylori infection is a risk factor for acute diarrhoea and persistent diarrhoea: a case control study.

Summary of Referee's Opinions: **Please see the following table to evaluate the various aspects of the proposal by checking the appropriate boxes. Your detailed comments are sought on a separate, attached page.**

	Rank Score		
	High	Medium	Low
Quality of Project		✓	
Adequacy of Project Design			✓
Suitability of Methodology		✓	
Feasibility within time period	✓		
Appropriateness of budget	Not indicated		
Potential value of field of knowledge	✓		

CONCLUSIONS

I support the application:

a) without qualification ☐

b) with qualification

- on technical grounds ☐
- on level of financial support ☐

I do not support the application ☑

Helicobacter pylori infection is a risk factor for acute diarrhoea and persistent diarrhoea: a case control study.

Comments:

Considerable thought and care has clearly gone into this proposal. The authors seem particularly concerned about the fact that it is of hospital based cases rather than community based. I have no problem with this particular aspect although if there is a positive finding one might wish to look again at the question using a community based study. The case definitions are very clear and the methods of determining exposure status to H. pylori seem adequate and appropriate.

There seem to be two major areas of concern with the proposal as it stands. These relate to; confounding - measurement and control, the form of analysis. First the question of confounding; this is clearly a major potential problem given the strong associations of both H.pylori, reduced gastric acidity and diarrhoea with a number of factors. Some of these are mentioned in the background document - low socio-economic status, large family size, crowding etc.. The adequate control of these presents a particular difficulty in this study. It is not clear whether, and how, they will be measured and controlled for. The choice of neighbourhood controls who are usual attenders of the clinic clearly controls for some of them, but how much? This choice of controls also has the potential to overmatch. We know so little about the epidemiology of H. pylori that it is possible that it clusters in neighbourhoods. In which situation any association would be obscured. My preference would be to choose controls at random from the catchment area of the clinic and to control for confounding by careful measurement and statistical analysis. Since clinic attendance may have some effect on the estimate of risk why not select two control groups; one random clinic attenders from the community and one completely at random.

The second issue that I have with confounding relates to age. It appears that the controls will be selected with two rather broad age bands. This could potentially result in marked age mis-matching within pairs. Since H. pylori prevalence may be rising very steeply across this age range this could present a problem. It is exacerbated by the neighbourhood controls since they necessitate a matched analysis - this may make age adjustment difficult with such crude age matching. One could end up discarding a lot of pairs from an age adjusted analysis.

The second problem is with the analysis. It is stated that this will be adjusted for age and sex. It is not stated that it will be by matched analysis, nor is the method stated. It is clear that conditional logistic regression would be most appropriate. This introduces all of the problems above. If random controls are selected then the analysis is much simpler and more robust.

Although this study is of great interest it will be difficult to feel confident that any result is not due to confounding. Perhaps the authors might consider a randomised intervention study against H. pylori with measurement of subsequent diarrhoea as the outcome. This removes the very difficult problem of confounding in this study.

Title: Helicobacter pylori infection is a risk factor for acute diarrhoea and persistent diarrhoea: a case control study.

Summary of Referee's Opinions: **Please see the following table to evaluate the various aspects of the proposal by checking the appropriate boxes. Your detailed comments are sought on a separate, attached page.**

	Rank Score		
	High	**Medium**	**Low**
Quality of Project		✓	
Adequacy of Project Design			✓
Suitability of Methodology			✓
Feasibility within time period			
Appropriateness of budget	-	-	-
Potential value of field of knowledge	✓		

CONCLUSIONS

I support the application:

a) without qualification ☐

b) with qualification

- on technical grounds ☐
- on level of financial support ☐

I do not support the application in its present form. ☒

HELICOBACTER PYLORI IS A RISK FACTOR FOR ACUTE DIARRHOEA AND PERSISTENT DIARRHOEA: A CASE-CONTROL STUDY

INTRODUCTION

* The authors of this research proposal should be congratulated on their roposal to study the relationship between *H. pylori* infection and diarrhoeal isease.

* Yet, both substance and form of the proposal in the latter's present state re not up to the mark. Before starting this type of case-control study, the uthors might consider some preparatory work such as (i) a serological survey (to etter delineate age-groups and to justify their case-control approach), and ii), if practicable, a combination of serology, endoscopy and breath analysis. One an not but wonder whether the authors have enough experience of breath analysis in oung infants (a rather tricky procedure!).

* One would also expect a typescript with a decent lay-out and without pographical errors and spelling errors.

* Anyhow, I feel the authors ought to be encouraged to vigorously pursue eir idea.

* The edited typescript is enclosed and some more detailed comments follow re.

BACKGROUND

(Page 1)

The paragraphs # 1 to 4 form the cornerstone of this project. Paragraphs # 1, 2, d 4 are adequate. Paragraph # 3 seems to imply that *H. pylori* is a 'host factor' ssibly explaining Dr. Baqui's findings. If this is correct, it should be clearly ated. Paragraph # 4 is more prudently and, I feel, more adequately phrased than e two following sentences.

(Pages 3 and 4)

The figures on the prevalence of *H. pylori* infection are informative, but I gest to add to each of them the 95% confidence intervals. The same applies to odds ratios on page 4 and to similar data throughout this typescript (annexes luded).

The last paragraph of page 4 merely repeats paragraph # 3, page 2. This might be place to elaborate somewhat on this interesting issue.

(pages 6 and 7)

The two paragraphs on the urea breath test could be incorporated in Annex 2 or,

HYPOTHESES

5. I have some problems here. I suppose that, in the present context, we can accept the following definition of the term hypothesis (see 'The Shorter Oxford English Dictionary'): "a provisional supposition which accounts for the known facts, and serves as a starting-point for further investigation by which it may be proved or disproved". Taken as such, the hypotheses are certainly interesting and the authors should be encouraged to pursue them. Yet, I submit that the planned research will NOT address any of the four hypotheses as they are stated.

1. The absence or presence of gastritis and hypochlorhydria will not be ascertained.

2. Hence, susceptibility to infection, colonisation, and diarrhoea, and the latter's duration, in the absence or presence of the two above conditions, cannot be determined.

3. Moreover, how will susceptibility be determined? If susceptibility is defined as an expression of the rate of a disease, one has to recall that Schlesselman (Case-Control Studies, page 18, Table 1.4) points out that one of the disadvantages of case-control studies is their inability to determine rates of diseases...

SELECTION OF CASES AND CONTROLS
(Pages 8 AND 9)

6. Group 1 children '...will be followed up at home...'. With which frequency? Group 2 children '...will be observed for sufficient duration...'. What is sufficient? Observed where? In the hospital or at home? Quite importantly, which treatment, if any, will be offered to the children? To which extent will treatment introduce a bias?

7. Both Group 1 and Group 2 children will be random samples. How will the randomization be done?

8. If the controls 'will be selected by two age strata...according to the age of the cases', the cases themselves should be selected in the same way. Indeed, the two groups will be compared with each other, and the ages of random samples of children with acute vs. persistent diarrhoea might differ. Also, a range from 3 to 17 months seems too broad to me because, amongst other things, it includes both fully breast-fed, partially breast-fed, and fully weaned children.

9. The selection of the controls seems awkward. Furthermore, there is no clear indication as to what the investigators plan to do with the controls, except for the statements that they will be submitted to a breath test (page 10, objective 3) and will not be bled (page 15).

OBJECTIVES
(Page 10)

10. The objectives are phrased as questions. Their content overlaps the hypotheses to a considerable extent but, the stress is now shifted from achlorhydria and gastritis to *H. pylori* infection, which is more appropriate. Would it still not be

11. Yet, I still fail to see how susceptibility can be determined. I do understand that, knowing (i) the prevalence of a potential risk-factor (*H. pylori* infection) in a population, and (ii) the prevalence of a particular condition (acute and/or persistent diarrhoea) in individuals with and without this risk-factor, one can compute a measure of susceptibility. But can the controls, as defined in this study be considered as a representative sample of a population? I hope I am wrong, but I have my doubts.

STUDY DESIGN
(Pages 10 to 18)

12. Since the authors do not state which treatment they will offer to their patients, ethical problems related to treatment can not be evaluated. Surely, case-control studies as well as cohort studies can be hampered by ethical problems .

13. I tend to agree with the choice of the confounding factors (pages 10 and 11), but why adjust for age and sex only in the analysis? Should one not control for them in the selection? Also, at what time of the study does one control for the other confounders?

14. I submit that, as a rule, investigators should indicate which software they use for all their statistics, including sample-size calculations.

15. In the paragraph on the urea breath test (page 15), it is said 'breath samples [will be] collected at 30 min and 40 min after administration of 13C urea [at a dose of 2 mg/kg]'. Yet, the Annex 2, page 3, states 'breath samples will be collected every 10 minutes for 1 hour'. Furthermore, the test-dose of 13C urea is now 5 mg/kg!. There seem to be a problem here.

16. Which meal will be given to delay gastric emptying time? It should be specified, and ought to be rich in fats.

17. As to the Analysis section, stratified chi-squares may offer interesting insights, but should one not take into account the impact of stratification on the sample size? The overall 2*2 tables might yield reliable results, but the strata will have smaller numbers and, hence, lack power.

18. One would prefer to see the form as it will be used and not just a list of variables.

19. Annex 1 is not without interest but is it really necessary?

20. Annex 2 could be incorporated in the body of the text.

Response to the reviewers' comments

Reviewer 1

1. Information about socio-economic status, family size, crowding, etc. will be gathered in a pre-designed and pre-tested questionairre form. This type of forms are being used in other similar studies in the ICDDR,B and has now been standardised. The points being noted include household possessions, quality of roof, floor and walls, number of rooms, and number of people sharing the same cooking pot, which have been found useful in defining the strata.

2. We accept the suggestion of including two groups of controls, i.e. one group of neighbourhood clinic attenders, and another community control group selected at random, after age stratification.

3. Age will be regarded as factor, and will be carefully controlled and adjusted for in the analysis phase. Because of this , the two age bands are kept relatively broad; otherwise over-matching will deter the use of age as a variable.

4. The analysis will not be matched - thus the question of mis-matched pairs will not arise. The data analysis plan is now provided in more detail.

5. At this moment, there is neither enough justification nor a practical and effective intervention against *H. pylori*; the suggestion of a randomised intervention study may be carried out in future.

Reviewer 2

Introduction :

A prelimenary survey in the ICDDR,B has shown that more than 50% of the sampled diarrhoeal children are H.pylori positive.

We have been collecting breath samples from children of all age groups including neonates for quite some time as part of different study protocols, and the techniques have now been standardised.

Background :

The data has been quoted as they had been provided in the papers, 95% confidence intervals were not provided to be qouted.

The paragraphs on the urea breath tests are now incorporated in Annex 2.

Hypotheses :

The hypotheses presented are based partly on facts and partly on logical and plausible deductions from current knowledge, and has been presented only as a suggested (and highly probable) model. The proposed study does not aim to address them which will need experimental studies; rather the objective is to examine the degree of association between diarrhoea and H. pylori infection as a possible risk factor, from a non-interventional epidemiological point-of-view. The points have been clarified in the text.

Selection of cases and controls :

Group 1 children wiil be followed up at home after 72 hours of discharge, only once, just to ascertain that diarrhoea does not last longer than 10 days.

Group 2 children will be observed at hospital. The reason for observation is to assure that they indeed have diarrhoea, and the total duration of diarrhoea is not less than 14 days; thus the period of observation will be upto fulfillment of these criteria.

As this is not an intervention trial, there is no need for randomisation.

The selection of controls have been modified and are explained more clearly.

Objectives:

As stated earlier, this study (with a case-control design) does not aim to prove or disprove the hypotheses - even a causal inference is difficult to draw without temporal association. A strong association will be looked for, after controlling the known confounders; rest of the inference will be based on analysis and interpretation.

Study Design:

Treatment to the patients is not a part of this study, and the patients will recieve the standard ICDDR,B treatment as befitting the clinical condition.

All possible confounders, including age and sex, will be adjusted during analysis.

The description of the urea breath test has been corrected.

Breast-fed children wiil be allowed breast-milk, others will recieve a standard milk-formula.

The questionaire form is provided.

HELICOBACTER AS A RISK FOR DIARRHOEA

Name of the child: _____

Father's name : _____

Address : _____

_____

_____

1. Serial # ___/___/___/

2. Hospital I.D. # ___/___/___/___/___/___/

3. Date of admission ___/___/___/___/___/___/
D D M M Y Y

4. Time of admission ___/___/___/___/

5. Age of the child (months) ___/___/

6. Sex of the child (male=1, female=2) ___/

7. Age of the mother (years) ___/___/

8. What is your religion ___/
(Muslim=1, Hindu=2, Christian=3,
Buddhist=4)

9. Distance covered (miles) ___/___/___/___/

10. Main mode of transport ___/

On foot=1, Rickshaw=2, Bus=3, Scooter=4,
Car=5, Boat=6, Tempo=7, Ambulance=8,
Other=9

11. Date of onset of diarrhoea ___/___/___/___/___/___/
D D M M Y Y

12. Time of onset of diarrhoea ___/___/___/___/

13. Date of onset of vomiting ___/___/___/___/___/___/
D D M M Y Y

14. Time of onset of vomiting ___/___/___/___/

15. Duration of acute diarrhoea ___/___/
(hours)

16. Duration of vomiting in case of acute diarrhoea (hours) ___/___

17. Duration of persistent diarrhoea (days) ___/___

18. Any sickness in last one month: None ___/ Cough ___/
(Yes=1, No=2)

Fever ___/ Cough and fever ___/ Fever, sneezing & running nose ___/

Repeated cough, fever & rapid respiration ___/ Mumps ___/

Measles ___/ Ear discharge ___/ Scabies ___/ Conjunctivitis ___/

19. Any diarrhoea in last one month: ___/ 20. If yes, duration in days ___/___/
(Yes=1, No=2)

21. Any diarrhoea in last three months: ___/ 22. If yes, duration in days ___/___/
other than # 14 & 15)
(Yes=1, No=2)

23. Prior treatment: none ___/ Antibacterial drug ___/
(Yes=1,No=2)

Anthelmentic drug ___/ Antiprotozoal ___/ Antiperistaltic ___/

Other ___/

24. Use of ORS at home ___/
(Packet=1,Salt-sugar=2,both=3))

25. Dehydration ___/
(Mild=1, moderate=2, severe=3)

26. Lower respiratory tract ___/
(Normal=1, Infection present=2)

27. Eyes ___/
(Normal=1, Infection present=2)

28. Ears ___/
(Normal=1, Infection present=2)

29. Throat ___/
(Normal=1, Infection present=2)

30. Skin ___/
(Normal=1, Infection present=2)

31. What was the gestation period of this child ___/___

32. Where did your delivery take place (house=1, hospital=2, clinic=3, other=4) ___/

33. Did you have any problems during delivery (Prolonged delivery pain, more than 24 hours=1, excessive bleeding=2, convulsion=3, High fever=4 ___/

34. Who attended your delivery (Doctor=1, Nurse=2, trained TBA=3, TBA=4, relative=4, friend=5, neighbour=6, self=7, multiple=8, other=9) ___/

35. Has the child been immunised:

DPT ___/
(Ist dose=1, Ist+2nd dose=2, Ist+2nd+3rd dose=3, none=4)

OPV ___/
(Ist dose=1, Ist+2nd dose=2, Ist+2nd+3rd dose=3, none=4)

BCG ___/
(Yes=1, No=2)

Measles ___/
(Yes=1, No=2, Not applicable=3)

36. Other than this sick child, how many children (living) do you have ___/

37. What is the birth order of this sick child ___/

38. Any sick person in the family other than this child (Yes=1, no=2) ___/

39. Is the child breast fed now (exclusively=1, exclusively + plain water=2, partially=3, no=4) ___/

40. If partially breast fed, since what month of the child ___/___

41. What was the main feed that you gave to begin with in addition to breast milk:

42. What other feeds you subsequently added in addition to breast milk:

43. Did you breast feed during baby's illness (yes=1, no=2) ___/

44. If yes, did he suckle at ease=1, needed to be persuaded=2, refused food totally=3 ___/

45. Feeding at the onset of present diarrhoea: BM ___/ Water ___/
(Yes=1, No=2)

Unbranded powder milk ___/ Commercial baby food ___/

Cow's milk ___/ Goat's milk ___/ Buffalo's milk ___/

Unbranded powder milk + suji ___/ Commercial baby food + suji ___/

Cow's milk + suji ___/ Goat's milk + suji ___/

Buffalo's milk + suji ___/ Solid ___/ Other ___/

46. Feeding before the onset of present diarrhoea: BM ___/ Water ___/
(Yes=1, No=2)

Unbranded powder milk ___/ Commercial baby food ___/

Cow's milk ___/ Goat's milk ___/ Buffalo's milk ___/

Unbranded powder milk + suji ___/ Commercial baby food + suji ___/

Cow's milk + suji ___/ Goat's milk + suji ___/

Buffalo's milk + suji ___/ Solid ___/ Other ___/

47.Discharge wt (Kg) ___/___/.___/___/ 48.Length ___/___/.___/

49.Tibial length ___/___/.___/ 50.Mid arm circumference ___/___/.___/

51.Skinfold Triceps ___/.___/___/ 52.Skinfold subscapular ___/.___/___/

53.Rectal temp (c) ___/___/.___/

54.Does your husband perform any other job (for cash or kind) besides primary occupation? (Yes=1, No=2) ___/

55.How much did he earn last month ___/___/___/___/___/

56.Is there any member give money to the family (yes=1, no=2) ___/

57.How much did he give last month ___/___/___/___/___/

58.Do you have a paid job or any work (service with monthly salary, small business, maid servant, day labour, garment worker) (Yes=1, No=2) ___/

59.If yes, how much did you earn last month ___/___/___/___/___/

60.What is the primary occupation of your husband ___/___/
(Farmer=1, Day labourer/share-cropper=2, Rickshaw/push cart puller=3, Taxi/bus/truck driver=4, Mill worker=5, Skilled worker (mason, carpenter barber, washerman)=6, Non-executive=7, Office executive=8, Petty business (earns less than 3000 taka a month)=9, Big business=10, Overseas employment=11, Boatman=12, Fisherman=13, Other=14, absent=15, dead=16, unemployed=17

61. Number of sleeping room in the household ___

62. What is the floor material of that sleeping room (Cemented=1, noncemented=2) ___

63. Do you have paid electricity (yes=1, no=2) ___

64. What assets do you have:

(yes=1, no=2)

- TV ___
- Radio/transistor ___
- Freeze ___
- Electric fan ___
- Wrist watch ___
- Cot luxury ___
- Cot ordinary ___

65. Education of mother ___ ___
what class did you reach
Can not read or write=88,
can sign only=89,
can read only=90

66. Education of father ___ ___
what class did you reach
Can not read or write=88,
can sign only=89,
can read only=90

67. Source of drinking water ___
Tubewell/tap in compound=1,
Tubewell/tap in other's compound=2,
Pond=3, dug well=4, canal=5,
river=6

68. Source of water for washing/bathing ___
Tubewell/tap in compound=1,
Tubewell/tap in other's compound=2,
Pond=3, dug well=4, canal=5,
river=6, ditch=7

69. Drinking water stored in container ___
(Pitcher=1, bucket=2, drum=3,
big earthen vessel=4, jug=5,
jerrycan=6, saucepan=7,
other=8

70. Water container:
(Covered=1, uncovered=2) ___/

71. Utensil mainly used for feeding main food
(Feeding bottle=1, cup=2, plate=3, spoon=4, bowl=5, mug=6, other=7 ___/

72. Before feeding the child, mother washes hand with:
(Water only=1, soil=2, soap=3, ash=4, other=5) ___/

73. After defecation, mother washes hand with:
(Water only=1, soil=2, soap=3, ash=4, other=5)

74. Toilet
Open place=1, pit=2, hanging=3, sanitary with flush=4, sanitary without flush=5 ___/

75. Presence of domestic animals
(Yes=1, no=2) ___/

76. If your child had diarrhoea where you would have taken him
(ICDDR,B=1, local practitioner=2, local health facility=3, Shishu hospital=4, DMCH/PG hospital=5, At home=6, other=7 ___/