Library   Date 31/7/95

Principal Investigator __Md. Yunus__   Trainee Investigator (if any) _____

Application No. __95-020__   Supporting Agency (if Non-ICDDR,B) __NIH__

Title of Study __Does disease due to Vibrio__
__olerae 01 confer protection against sub-__
__quent diarrhoea due to V. cholerae 0139__
__an analysis of the existing data__.

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).

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 NA
(b) Social Risks   Yes No NA
(c) Psychological risks to subjects   Yes No NA
(d) Discomfort to subjects   Yes No NA
(e) Invasion of privacy   Yes No NA
(f) Disclosure of information damaging to subject or others   Yes No NA

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 NA
(b) Procedures to be followed including alternatives used   Yes No NA
(c) Physical risks   Yes No NA
(d) Sensitive questions   Yes No NA
(e) Benefits to be derived   Yes No NA
(f) Right to refuse to participate or to withdraw from study   Yes No NA
(g) Confidential handling of data   Yes No NA
(h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure   Yes No NA

5. Will signed consent form be required:
(a) From subjects   Yes No NA
(b) From parent or guardian (if subjects are minors) Yes No NA
6. Will precautions be taken to protect anonymity of subjects   Yes No NA
7. Check documents being submitted herewith to Committee:
____ Umbrella proposal - Initially submit ar 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
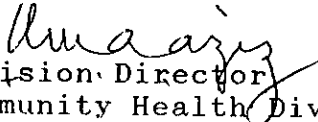1. A description of the areas to be covered in the questionnaire or interview which could be considered either sensitive or which would constitute an invasion of privacy.
2. Examples of the type of specific questions to be asked in the sensitive areas.
3. An indication as to when the questionnaire will 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.

__Md. Yunus__
Principal Investigator

_____
Trainee

1. Investigators   : Md. Yunus, John Clemens*, K Zaman

2. Title of Project   : Does disease due to <u>Vibrio</u> <u>cholerae</u> 01 confer protection against subsequent diarrhoea due to <u>Vibrio</u> <u>cholera</u> 0139 ? - an analysis of <u>the existing</u> data.

3. Starting date   : As soon as the protocol approved

4. Date of completion   : 12 months from the start of the protocol

5. Total Budget Required : US$ 10,000/-

6. Funding souce   : National Institute of Health

7. Division Director   : Division Director
                                 Community Health Division

*National Institute of Child Health and Human Development, National Institute of Health, USA

ABSTRACT

Recently a non-01 Vibrio cholerae organism designated as V.cholerae 0139 has emerged as a cause of epidemics of cholera-like diarrhoeal disease in India and Bangladesh, and has spread to several neighbouring countries with potential for pandemic. The disease has primarily affected adults which suggests that the population lacked natural immunity to this organism. A case-control study is proposed to assess whether an initial treated episode of diarrhoea due to V.cholerae 01 was associated with a reduced risk of subsequent treated episode of diarrhoea due to V.choleare 0139, during a period of up to ten years following the initial episodes. "Cases" will be Matlab DSS residents with diarrhoea associated with isolation of V.cholerae 0139, detected in Matlab diarrhoea treatment centre during 1993 and 1994. "Controls" will be subject who did not develop this outcome during 1993 and 1994, and will be matched to the cases by age, sex, neighbourhood (bari) of residence at the time that the case became ill, and duration of residence in Matlab.

Cases and Controls will be contrasted for the comparative occurrence of an earlier episode of treated V.cholerae 01 diarrhoea during the period 1983-92 using odds ratios appropriate for matched data. 95% confidence intervals will be estimated for these odds ratios using test-based methods, or exact techniques when mandated by sparse data. Adjustment of the crude odds ratios for potentially confounding variables that are unequally distributed between cases and controls will be undertaken with conditional logistic regression modes, which are designed to account for the matched selection strategy of cases and controls. 95% confidence intervals for these adjusted odds ratios will be estimated with use of standard errors of the coefficients. The results of this study would have important implications for future cholera vaccine development and cholera control.

8. STUDY OBJECTIVES:

a) Primary

1. To assess whether an initial treated episode of diarrhoea due to V.cholerae 01 was associated with a reduced risk of a subsequent treated episode of diarrhoea due to V.cholerae 0139, during a period of up to ten years following the initial episode.

b) Secondary

(i) To evaluate whether the relationship in 1) was modified by the biotype of the initial episode.

(ii) To ascertain whether the relationship in 1) was modified by the recency of the initial episode.

(iii) To assess whether the relationship in 1) was modified by the age at the time of the initial episode

9. SIGNIFICANCE

Cholera is a major public health problem in Bangladesh and many other developing countries. Epidemics of cholera due to Vibrio cholerae 01 regularly occur in Bangladesh leading to significant morbidity and mortality. The appearance of the new serogroup of cholera designated as V.cholerae 0139 in late part of 1992 has posed an additional threat to life. The V.cholerae 0139 outnumbered the V.cholerae 01 during 1993 but in 1994 V.cholerae 01 took the lead again although both types were simultaneously present. That the V.cholerae 0139 has predominantly affected the adult population indicates no cross protection offered by V.cholerae 01 against 0139 cholera. This empiric observation needs to be scientifically validated which would have important implications for future cholera vaccine development and cholera control. The proposed study is expected to contribute in this regard.

10. ETHICAL IMPLICATIONS

The proposed study would use routinely collected hospital data on cholera patients at Matlab Diarrhoea Treatment Centre, data of 1985 cholera vaccine trial project and some demographic information from the existing Matlab DSS records.

No new data collection is planned for this project. Therefore, there is no ethical questions involved in this study.

11. BACKGROUND, RESEARCH PLAN AND BIBLIOGRAPHY

Background

In October 1992 an epidemic of a cholera-like diarrhoeal illness due to a non-01 <u>Vibrio cholerae</u> organism was recognized in Madras, India. During the ensuing six months, this epidemic spread with great rapidity to both the east and west coasts of India, and to both coastal and inland areas of Bangladesh [1,2,3]. The course of the epidemic has been exceptionally aggressive resulting in large number of cases and deaths. By the end of March, 1993, the Government of Bangladesh Epidemics Surveillance had reported 107,297 cases of diarrhoea and 1473 deaths (4). Moreover, during this epidemic typical <u>Vibrio cholerae</u> 01 disease virtually disappeared, suggesting ecological displacement of <u>Vibrio cholerae</u> 01 by the new epidemic organism. Microbiological analysis of the epidemic organism has revealed that it belongs to none of the 138 recognized <u>Vibrio cholerae</u> serotypes, though it produces cholera toxin identical to that produced by <u>Vibrio cholerae</u> 01 and in many respects resembles the El Tor biotype. This new organism has therefore been classified as <u>Vibrio cholerae</u> 0139 (Bengal) [3].

The descriptive epidemiological features of this illness have been evaluated by ICDDR,B scientists, both in the Dhaka treatment centre and in the Matlab field studies area. Perhaps the most noteworthy epidemiological features of the epidemic are that it has primarily affected adults (5,6). This pattern contrasts directly with the pattern noted for endemic cholera, for which the risk is highest for young children over two years of age, and adult females [7]. Because the latter pattern is thought to be in part due to age related acquisition of natural immunity from infections by <u>V.cholerae</u> 01, the pattern seen with <u>V.cholerae</u> 0139 suggests that the populations affected by the epidemic of <u>V.cholerae</u> 0139 lacked natural immunity to this organism, despite having considerable immunity to <u>V.cholerae</u> 01.

If this explanation for the age-related occurrence of <u>V.cholerae</u> 0139 is true, it suggests that infection by <u>V.cholerae</u> 01 does not confer immunity against infection by <u>V.cholerae</u> 0139. It also suggests that available inactivated and live vaccines against <u>V.cholerae</u> 01 will not confer substantial protection against <u>V.cholerae</u> 0139 disease. Because an absence of cross-protection by <u>V.cholerae</u> 01 against <u>V.cholerae</u> 0139 would have extremely important ramifications for future cholera vaccine development, and because evidence for an absence of such protection is at present indirect, it is important that studies be undertaken to evaluate this possibility in a direct fashion.

Past work in the Matlab field studies area of ICDDR,B has demonstrated the value of assessing the immunizing potential of natural cholera episodes by evaluating the risk of recurrent episodes in relation to the risk of initial episodes [7,8].

Such studies have demonstrated that natural <u>V.cholerae</u> 01 disease is indeed immunizing against recurrent disease by this serotype, though the protection conferred by an initial episode of classical cholera may be greater than that conferred by an initial episode of El Tor cholera [8].

The present study will use the same approach to test whether an earlier episode of <u>V.cholerae</u> 01 diarrhoea was associated with a reduced risk of <u>V.cholerae</u> 0139 diarrhoea during the 1993 and 1994 epidemics in Matlab. The availability of the computerized demographic surveillance data of the Matlab population through the Matlab Demographic Surveillance System (DSS) [9], together with the long-term comprehensive surveillance system for cholera in Matlab hospital, make Matlab a uniquely appropriate site for performing this important study.

RESEARCH PLAN

Study Hypothesis:

We hypothesize that antecedent disease caused by <u>V.cholerae</u> 01 provides no protection against subsequent diarrhoeal illness caused by <u>V.cholerae</u> 0139 as adults have been primarily affected by <u>V.cholerae</u> 0139.

METHODS:

This study would use routinely collected Hospital data on cholera patients at Matlab DTC, data of 1985 cholera Vaccine trail project and socio-demographic information from the existing Matlab DSS records.

1) Overview

This evaluation will be undertaken as a case-control study. "Cases" will be Matlab DSS residents with diarrhoea associated with isolation of <u>V.cholerae</u> 0139, detected in the Matlab treatment centre during 1993 and 1994. "Controls" will be subjects who did not develop this outcome during 1993 and 1994, and will be matched to the cases by age, sex, neighbourhood (bari) of residence at the time that the case became ill, and duration of residence in Matlab. Cases and controls will be contrasted for the comparative occurrence of an earlier episode of treated <u>V.cholerae</u> 01 diarrhoea during the period 1983-92. Because of the rarity of treated <u>V.cholerae</u> 0139 diarrhoea during 1993 and 1994, in relation to the total population at risk in Matlab (less than 5 cases per 1000), the odds ratio for the association will approximate the relative risk of <u>V.cholerae</u> 0139 diarrhoea in subjects with an earlier episode of treated <u>V.cholerae</u> 01 diarrhoea versus subjects lacking such a history. Thus, the expression [(1-odds ratio) X 100%)] will estimate the percentage protective efficacy conferred by an initial <u>V.cholerae</u> 01 episode.

ii) **Definition and Selection of "Cases"**

"Cases" will include any resident of the Matlab DSS area who presented for care of diarrhoeal episode from which V.cholerae 0139 was isolated. In this connection, diarrhoea will consist of a complaint of ≥ 3 loose or liquid stools during the 24 hours before presentation for care at Matlab diarrhoeal treatment centre, or presentation for care of an illness with ≥1 or an unknown number of loose or liquid stools but with an impression of moderate to severe dehydration on initial clinical examination. Treatment "episodes" of diarrhoea will be demarcated by ≥7 days between the onset of diarrhoea before the presentation for care of diarrhoea and discharge for the previous treatment encounter. For each case the following information will be collected from the hospital sheet and the DSS books: Name, Current Identification number (CID), Registration Identification number (RID) date of birth, date of admission, sex, religion, clinical information regarding type of diarrhoea and its management, date of entry into DSS.

iii) Definition and Selection of "Controls"

For each case, 3 "controls", matched to the case on the basis of age group, sex, bari (cluster of households) of residence, and duration of residence in the Matlab DSS area. In this connection, "age" will denote the age of the case in years on the day of presentation for care for the index episode, and the age of the three matched controls on the same date, and age groups will be demarcated into three groups: 0-4 years, 5-14 years, and 15+ years. "Bari of residence" will refer to the bari of residence on the date of presentation for care of the index episode for the case and the controls matched to the case. If three suitable matched controls cannot be found from the bari of residence of the case, then the next bari in the DSS census will be inspected; if three controls cannot be located in this way, the previous bari in the census will be inspected, and so on. Conversely, if more than three potentially eligible controls are identified in the bari of the case, three will be selected using a random number table. "Duration of residence" will refer to the duration of residence in the Matlab Demographic Surveillance area. Matching on this variable will be done on the basis of their duration of stay in the Matlab DSS area i.e controls will include only those persons who have resided in the DSS area for at least as long as the matched case. All controls for the study will be unique (e.g., will be sampled without replacement).

iv) **Ascertainment of Earlier V.cholerae 01 Episodes**

All microbiologically confirmed episodes of V.cholerae 01 diarrhoea detected among DSS residents at the Matlab treatment centre between January 1, 1983 and December 31, 1992 will be computerized (such episodes are already computerized for the interval January 14, 1985 – June 30, 1990). Such episodes will be defined with criteria identical to those for V.cholerae 0139 diarrhoea (vide supra),

except for the stipulation that V.cholerae 01, of either biotype and of either serotype, was isolated.

Linkage of these histories of earlier episodes to cases and controls will be performed by an automated, computerized algorithm, according to the unique registration identification number assigned by the Matlab DSS. As a secondary confirmation, it will be ascertained that the age noted on routine clinical examination at the time of the initial episodes corresponded to the DSS age of the case or control at that time within the following categories (0-4 years, 5-14 years, 15+ years) and that the patient treated for the initial episode was of the same gender as the linked case or control.

A case or control will be considered "exposed" to an earlier episode of V.cholerae 01 diarrhoea if these three linkage criteria (RID, age category, gender) are fulfilled.

v) Analysis of the Data

Cases will be contrasted with controls for the occurrence of an earlier episode of V.cholerae 01 diarrhoea using odds ratios appropriate for matched data. During analysis we shall censor antecedent histories of Cholera in the controls to the duration of residence of the matched case. For example, if the case had resided in the DSS area for only five years before the episode of 0139 Cholera, histories of Cholera for controls matched to the case would only be analyzed for the five years prior to the date of admission of the case (regardless of how long the controls had been resident in the DSS area)

With this strategy, it will not be necessary to exclude cases under 10 years of age or persons who have migrated in after 1983. As already noted, these odds ratios will approximate the risk ratios of subsequent V.cholerae 0139 diarrhoea in persons with versus those without an earlier episode of V.cholerae 01 diarrhoea, 95% confidence intervals will be estimated for these odds ratios using test-based methods, or exact techniques when mandated by sparse data [10]. Analysis of distinctions in associations created by the biotype of the initial episode, as well as by the recency of the initial episode and the age at the time of the initial episode, will employ conventional techniques for evaluating trends of odds ratios in case-control studies [10].

Adjustment of the crude odds ratios for potentially confounding
variables that are unequally distributed between cases and controls
will be undertaken with conditional logistic regression models,
which are designed to account for the matched selection strategy of
cases and controls [10]. In these models, case-control status is
taken as the dependent variable, and exposure to an earlier episode
(coded as a yes/no binary variable) and other covariates are fitted
as independent variables. The exponential of the coefficient for
the exposure variable in such models represents the odds ratio for
the association, adjusted for other independent variables included
in the model. 95% confidence intervals for these adjusted odds
ratios will be estimated with use of the standard errors of the
coefficients.

vi) **Sample size Justification**

This study is framed as an "equivalence study", in that we suspect
that an initial episode of V.cholerae 01 diarrhoea will confer no
protection against a subsequent episode of V.cholerae 0139
diarrhoea. If, as predicted, we fail to find a protective
association between these two infections, we want the results of
the study to exclude the possibility of substantial protection with
reasonable confidence. Accordingly, the method of Blackwelder [11],
adapted to case-control studies, is used with the following
assumptions: 1) 2% of controls will have had an episode of treated
V.cholerae 01 diarrhoea during the 10 years before selection (12);
and 2) given no true difference in the prevalence of antecedent
V.cholerae 01 episodes in cases vs. controls, we wish the one-
tailed 95% confidence interval for the overall odds ratio to
exclude ≥ 80% protection with ≥ .8 power. The sample needed to meet
these assumptions is 626 cases, with 3 controls for each case, for
a total of 626 X 4 = 2504 subjects.

12. **BIBLIOGRAPHY:**

1. Albert M, Siddique A, Islam M, et al. Large outbreak of clinical cholera due to Vibrio cholerae non-01 in Bangladesh (Letter). Lancet. 1993; 341:704

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10. Breslow N, Day N. Statistical Methods in Cancer Research. Volume 1: The Analysis of Case-Control Studies. IARC Scientific Publications No.32. Lyon: 1980.

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12. Yunus M, Siddiqure AK, Sack RB, Epidemiology of Cholera in Bangladesh. The proceedings of the 28th joint conference, U.S - Japan co-operative Medical Science program, Cholera and related diarrhoeal disease panel,
Tokyo, July 20 - 21, 1992, pp 70 - 75

End

BUDGET

ITEMS	PERSON-MONTH	RATE/MONTH	TOTAL
Dr. Md. Yunus 5%	12	US$ 95.00	1,138.00
Sr. Health Assistant	18	US$ 387.00	6,966.00
Sr. Field Research Officer 5%	12	US$ 33.00	396.00
Supplies and Materials			1,000.00
Miscellaneous expenses			500.00
		Total:	10,000.00

30/7/95

Title: Does disease due to Vibrio cholerae O1 Confer Protection against subsequent diarrhoea due to Vibrio cholerae O139? - an analysis of the existing data.

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   [ ]

Name of Referee:

Signature:.....................Date: ....

Position:
Institution:

Detailed Comments

Comments on the protocol "Does disease due to <u>Vibrio</u>..."

The project has a well defined objection, an appropriate methodology and a clear rationale/significance. The budget is low since it uses existing data. There are nevertheless a few technical aspects that need improvement. In particular:

1. Cases cannot be "any resident of the Matlab DSS area...." In particular they must have been alive and residing in the DSS in 1983; otherwise their exposure status is unknown. Note that this rules out children under 10 and inmigrants since 1983. The investigators can check these variables for potential cases first.

2. Use of some SES data would seem appropriate. In particular, water source and toilet facility available to the household. Others are also available I believe.

3. The covariates of residence in village abutting a river or with a weekly bazaar are wrong because village is matched!

4. The sample size text is terse. In particular:

a) What is the reference for level of .02
b) The formula is different if there are 3 controls per case. See text of Fleiss.

A minor comment. It seems inappropriate to have 18 months time for the Health Asst. if the others are only working 12 months on the project. Or does this involve two Health Assts? Clarify.

Title: Does disease due to <u>Vibrio cholerae</u> 01 confer
          protection against subsequent diarrhoea due to
          <u>Vibrio cholerae</u> 0139? – an analysis of the existing
          data

<u>Summary of Referee's Opinions:</u> 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		X	
Adequacy of Project Design		X	
Suitability of Methodology		X	
Feasibility within time period	X		
Appropriateness of budget	X		
Potential value of field of knowledge		X	

<u>CONCLUSIONS</u>

I support the application:

        a)   without qualification   [   ]

        b)   with qualification   [X   ]   see letter

              -   on technical grounds   [   ]

    -   on level of financial support   [   ]

I do <u>not</u> support the application   [   ]

Name of I

Signature

Position:

Instituti   Dr. Md Yunus

Detailed

June 27, 1995

Dear Dr Aziz:

This is in response to your request for my review of the protocol **"Does disease due to Vibrio cholerae O1 confer protection against subsequent diarrhoea due to Vibrio cholerae O139? an analysis of the existing data."**

This is a case control study in which the cases will be those who developed cholera due to O139 and the controls will be age matched persons from the same or nearby bari's who did not. The crucial analysis will be to determine if the controls had a higher rate of previous O1 cholera, suggesting that they were protected from the O139 epidemic.   In general I find this to be an excellent proposal which will yield important information, though the evidence is already strong that one infection does not protect against the other serotype. My only suggestions are to consider two points in the analytical strategy.

1. Based on my reading of the sample size calculations which use one tailed assumptions, the analysis does not consider the possibility that previous O1 cholera might actually increase the risk for O139 cholera.   If, for a moment, we assume that there is no biological protective effect from an earlier O1 episode on the risk of a subsequent O139 episode, there could in fact be an observation of increased rates of O139 in persons who previously had O1, using the methods described in this study. This is possible if, for example, other risk factors such as low gastric acid, blood type, poor hygiene practices, or other unknown risk factors, placed an individual at higher risk of cholera in general. Then the individual would be at high risk of O1 and also at higher risk of O139, than his neighbors who do not have this risk factor. One of these factors (blood type) may be known from the data set, but others may not be known.

In other words, the predisposing factors for cholera are highly correlated with both serotypes and would tend to show that O1 increased the risk of O139. By contrast, the potential immunological protection (if it exists between O1 and O139) would decrease the risk for O139 if O1 had occurred previously. Which of these competing risks will be predominant and will this analysis be able to dissect these risks?

2. The age groups 0 - 4 may be a problem. This is a narrow age group in years, but a wide one in terms of risk. The period from 0-2 has nearly no risk, then it goes to maximum risk (for O1 cholera) from age 2 - 4. Lumping these two diverse age groups will likely dilute any effect or at least lead to possible misinterpretation.

I hope this review is helpful.

RESPONSES TO EXTERNAL REVIEWERS' COMMENTS

<u>Responses to External Reveiwer No. 1</u> (Responses are in order of the reviewer's points):

1) The reviewer is correct in asserting that cases and controls must have equal durations of pre-selection residence in Matlab; if cases and controls differ in this respect, their opportunity to have had earlier episodes of <u>V. cholerae</u> 01 diagnosed would also differ, creating a potential bias in the assessment. However, in contrast to the reviewer's statement, it is not necessary that cases and controls have a complete 10-year antecedent residence in Matlab for ascertaining histories of earlier cholera episodes. It is only necessary that the period of antecendant residence be equal for the case and the controls matched to the case. We now have modified our selection strategy to inclujde only those controls who have resided in Matlab for at least as long as the matched case (see p-6: Definition and selection of controls); we also have now modified our analytic strategy to censor antecedent histories of cholera in the controls to the duration of residence of the matched case (see p-7: Analysis of the data). For example, if the case had resided in Matlab for only five years before the episode of 0139 cholera, histories of cholera for controls matched to this case would only be analyzed for the five years prior to the date of admission of the case (regardless of how long the controls had been resident in Matlab). With this strategy, it will not be necessary to exclude cases under 10 years of age or persons who migrated in after 1983.

2) The published literature on cholera in Matlab (see Glass et al. Endemic cholera in rural Bangladesh, American Journal of Epidemiology. 1982; 116: 959 – 70) identifies only age, sex, religion, residence near a river, and residence near a bazar as sociodemographic determinants of the risk of cholera. Source of water (eg, presence of a tubewell) has been a notoriously disappointing variable in terms of its ability to predict the risk of cholera. Similarly, site of defecation has not proved to be a useful predictive variable in analyses of cholera risk in analyses of data from the 1985 field trial of oral cholera vaccines in Matlab (clemens, unpublished data). In any event, since cases and controls will be matched on bari of residence, this matching strategy <u>per</u> <u>se</u> should closely match cases and controls for virtually all socioeconomic and hygienic characteristics.

3) We agree that the matching strategy for cases and controls will automatically match the two groups for residence near a river and residence near a bazar (just as this strategy will match the two groups for other sociodemographic characteristics see our response to the reviewer's comment # 2). Therefore, we have omitted this section.

4) The reference for a prevalence of earlier 01 cholera of .02 is based on the fact that the average annual incidence of 01 cholera during the period in question (1983 - 93) was about 3 cases per 1,000 per year (see glass et al. Ref. # 7, and Yunus et al. Ref. # 12). 3/1,000 multiplied by 10 years yields a prevalence of 3%; to account for the minority of cases having less than 10 years residence in Matlab (see our response to reviewer's comment # 1), we reduced this prevalence to 2% in our calculation of sample size. Our sample size calculation is based on the formula for equivalence studies provided by Blackwelder and cited in the references; our calculation takes into account the use of 3 controlos per case. The inclusion of the additional formula in the text of our original protocol was in error, sice it is a formula designed for studies that seek to detect differences, rather than equivalence, which is the hypothesis of our study. The additional formula has now been omitted from the text. The text of Fleiss recommended by the reviewer is similarly inappropriate, since it only provides sample size formulas for studies that are designed to detect differences.

Sr. Health Assistant's time has been shown as 18 person-month. More than one Sr. Health Assistant will be involved.

Responses to Reviewer No. 2 (Responses are in order of the reviewer's points):

1) The reviewer perceptively suggests that unmeasured risk factors may be shared by 01 and 0139 infections, tending to create a direct risk rather than a protective association between them. We agree that this is a potential limitation of our analysis since we do not have information about such potentially confounding variables as ABO blood group and gastric acidity. However, we would point out that this same limitation has applied to past studies in Matlab addressing whether 01 infections confer protection against reinfections. Such analyses have consistently demonstrated protective associations, despite this potential bias suggesting that the mgnitude of the bias is likely to be minimal.

We will include a discussion of this potential limitaiton in the discussion section of the paper describing the findings of this study.

2) The reviewer's concern will be addressed by controlling for exact age in multivariate analyses to remove the residual confounding of using too broad an age range for selection of young children.