ETHICAL REVIEW COMMITTEE, ICDDR,B.

21

Principal Investigator Dr. W.M. Spira — Trainee Investigator (if any) _____

Application No 81-049 and Dr. M.I. Huq — Supporting Agency (if Non-ICDDR,B) _____

Title of Study Inter-village transmission of cholera in Matlab during the Epidemic ion. — 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 N.A.
   (b) Procedures to be followed including alternatives used — Yes No N.A
   (c) Physical risks — Yes No N.A
   (d) Sensitive questions — Yes No N.A
   (e) Benefits to be derived — Yes No N.A
   (f) Right to refuse to participate or to withdraw from study — Yes No N.A
   (g) Confidential handling of data — Yes No N.A
   (h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure — Yes No N.A
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 N.A.
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.

Environmental samples (water) to be entrained from affected areas & Control areas.

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

81-049
Rcvd : 8-12-81

SECTION I - RESEARCH PROTOCOL

1. Title : Inter-village transmission of cholera in Matlab during the Epidemic Season

2. Principal Investigators : Dr. William M. Spira and Dr. Md. Imdadul Huq

   Co-Investigator : Dr. John V. Lee

3. Starting Date : January 1, 1982

4. Completion Date : December 31, 1982

5. Total Direct Cost : US $ 22,829.00

6. Scientific Program Head :

This protocol has been approved by the Disease Transmission Working Group.

Signature of the Scientific Program Head : A Samad

Date : 10/11/1981

7. Abstract Summary :

In recent years epidemiological researches have been focussed to see how cholera spreads and where it resides during an inter-epidemic period. The proposed protocol on the inter-village transmission of V. cholerae to be carried out in Matlab aims at to answer the question how Vibrio cholerae 01 passes from one village to another during epidemic season and to identify the pattern of this transmission. The influence of weather conditions and hydrology in the area will be seen by both sattelite photographs as well as laboratory monitoring

etc. The clusters of V. cholerae isolated from the villages under study will be studied further in the laboratory by Phage typing, Plasmid screening, Restriction enzyme mapping, GLC and Exoprotein profile analyses of the isolates obtained from the study.

This will be a collaborative project between John Hopkins, Maryland and ICDDR,B and will not involve any human subject for study. Laboratory works will be shared between laboratories in Maryland and Dacca.

8. Reviews :

a. Research Involving Human Subjects : _____

b. Research Review Committee : _____

c. Director : _____

d. BMRC : _____

e. Controller/Administrator : _____

SECTION II - RESEARCH PLAN

A. Introduction

1. Objective: Our first objective is to show unambiguously how Vibrio cholerae 01 passes from one village to another during the post--monsoon cholera epidemic period in rural Bangladesh. Specifically, we will examine the hypothesis that intervillage cholera transmission is via open waterways flowing through this region. If this can be proved, our second objective will be to identify patterns in this transmission. We will then evaluate the extent to which these patterns, and, ultimately, cholera seasonality, are influenced by weather conditions and hydrology taking into account others factors such as temperature, dissolved oxygen, salt concentration, presence of algae and other planktons etc.

2. Background: One of the most intriguing issues remaining to be examined concerning the occurence of El tor cholera in Matlab is that of seasonality and the fact that the start of the cholera season appears to be marked by the appearance of the disease in several villages over widely scattered areas, suggesting multiple simultaneous introductions. The spread of cholera through the region is, as yet, uncharacterized, but it suggets no obvious pattern. Similarly unexplained is why cholera disappears with the onset of the winter months.

   This seasonal nature of cholera and the lack of pattern in its spread have suggested to some that epidemic V. cholerae strains have an environmental reservoir. Thus, a post-monsoon bloom of V. cholerae in the region's waters account for the sudden appearance of multiple cases and the apparent lack of patterns in the spread of cholera. Similaryl the environmental reservoir would account for cholera's persistence in the area even though few human cases can be found during several of the months between seasonal peaks.

One line of evedence that argues against this hypothesis is Dr. M.U. Khan's data that persons using river and canals have a higher rate of cholera than persons using only isolated tanks. If epidemic V. cholerae are autochthonous in aquatic environments, why should tank users be protected? Previous studies at the CRL by Drs. McLaughlin and Spira found the microflora and plankton, as well as physio-chemical properties, to be substantially similar in these two types of water systems. Dr. Khan's findings suggest that cases arise from exposure to water-borne V. cholerae shed upstream by earlier cases.

The main problem in examining the question of inter-village cholera transmission is that it has not been possible to identify which cases were in the same chain of transmission. Assuming that several cases did occur simultaneous in the early post-monsoon season, these could easily lead to multiple overlapping transimission pathways that would be impossible to isolate from one another. This alone could create the appearance that no consistent pattern existed and could obscure waterborne transmission between villages.

The only published data on this aspect is that of McCormick et al: Endemic cholera in rural East Pakistan (Now Bangladesh), Am. J. Epidem. 89:393-404, 1969, which focusses of classical cholera. McCormick et al claim (and this claim has been echoedby others) that the localtion of cholera cases during the first six weeks of the 1964-65 cholera season did not suggest spread from village to village.

These cases are, until week six, clusters either at the southern or northern end of the study area. The central region remained free of cholera during this period.

In Figure 1, the cases discussed are plotted on a larger map of the Matlab study area. They are seperated into two clusters, Northern and Southern on the basis of the geographic separation that existed through the fifth week. The cases in the central region, all of which occurred in week six have been assigned to the Northern cluster. This will be discussed further in the following analysis.

An examination of the two clusters suggested that a trend consisting of cases moving in a southerly direction with time might exist. This is important because such a trend would suggest that village to village

transmission could be following the general direction of water flow in the water region.

The following simple (and simplistic) analysis of these data suggests that the southerly trend in cases with time is statistically significant Each village shown in Figure 1 was ranked within its cluster on the basis how far North it lay on the map. This was determined by going from top to bottom of the map with a straight-edge held across the page. As the midpoint of each village appeared above the line, the number of the village was recorded. When two villages were at the same level, a left to right ranking was arbitarily used. Once each village was ranked, the week in which its first cholera case occurred was noted. The weeks were then translated into ranks. The paired ranks were then analyzed using the Spearman rank correlation co-efficient. This analysis is given Table 1. The trend in each cluster of villages was statistically significant.

This analysis is fairly straightforward except for two points. Village VS2 which had the only case in the first week of the season and another five cases in week six was not included in the analysis on the premise that this first case was too geographically isolated from any other during the following four weeks to justify being included in the cluster. The more important point is that the cases in the central region during week six were placed in the Northern cluster. This was done because the trend to this point, at least in the Southern cluster, was for a southerly movement of cases.

In the end, this analysis shows only: 1) that highly significant patterns in transmission can be identified/certain cases are assumed to be in the same line of transmission: and 2) that the assumptions made to obtain these clusters of cases are not at all unrealistic. The heart of the problem is that one can validly create case clusters on the basis of

Table 1. Location of villages on North-South axis versus time to first introduction of cholera.

Northerly rank	Village	Week of cholera introduction	Rank of week
(Southern cluster)			
1	V7	3	3.5
2	V14	3	3.5
3	V8	4	6.5
4	G	5	11.0
5	I	4	6.5
6	V9	2	1.5
7	V6	6	16.0
8	F	4	6.5
9	V5	2	1.5
10	U	4	6.5
11	A	5	11.0
12	V1	6	16.0
13	X	5	11.0
14	D	6	16.0
15	W	5	11.0
16	B	5	11.0
17	V10	6	16.0
18	V11	6	16.0
		rs = .6357	P .01
(Northern cluster)			
1	V35	5	4.0
2	V33	4	1.5
3	P	6	8.5
4	N	5	4.0
5	O	5	4.0
6	V29	4	1.5
7	K	6	8.5
8	V25	6	8.5
9	V18	6	8.5
10	V26	6	8.5
11	L	6	8.5
		rs = .6606	P .05

transmission patterns or one can describe transmission patterns on the basis of known case clustering, but one cannot do both. With the epidemiologic data currently obtained about cholera in Matlab, however, this is what would be required in order to describe its spread through the region.

One way to get around this problem would be to provide an alternate basis for clustering cases. The approach we propose is described in the following sections.

3. Rationale: The typical post-monsoon cholera period in the Matlab area appears to begin with multiple introductions of V. cholerae followed by an increasing number of cases that overlap in space and time. Causal links between cases in different neighborhoods or villages cannot usually be assigned on the basis of epidemiologic criteria. In this situation, it is not possible to determine precisely how cholera is spread through the region if there is any functional relationship between its spread and environmental factors, such as the hydrologic features of the area.

Through the use of highly specific sets of markers derived by phage typing plasmid screening, selected biochemical tests, exoprotein electrofocussing profiles and gas chromatography, it should be possible for us to place all isolates from the study period into groups that represent a lineage (members of the set of direct lineal descendants of the same organism). The epidemiologic pattern in Matlab suggests that the post-monsoon cholera epidemic peak probably consists of several or more such lineages being transmitted through the same general area at the same time. By clustering cases on the basis of lineage, it should be possible to separate the transmission pathway for each cluster from the other transmissions occurring simultaneously and describe its unique pattern in space and time.

Once described, the characteristics of such pathways can be compared and common patterns can be identified. The importance as risk factors of propinquity and/or sharing a flowing water source with previous cases can be evaluated to gain further insight to the mechanism by which cholera is spread. Finally, changes in the pattern of transmission with the progression of the season can be documented and may suggest functional relationships

with changes in environmental factors, such as hydrology, that can be subjected to further analysis.

3. Specific Aims

1. Identify all cases of cholera or diarrhea associated with V. cholerae 01 coming to the Matlab Hospital, Matlab OPD or other ICDDR,B clinics in the Matlab study area. Obtain an accurate record of time and location of each case.

2. Monitor hydrologic and other characteristics (depth, flow direction and rates, tides, salts concentration, presence of algae and planktons etc. of major waterways in the region and monitor weather conditions. (temperature rainfall) during the post-monsoon cholera period under study. Prepare maps of the study area showing flowing water systems and their location with respect to villages.

3. Prepare stock cultures of each V. cholerae 01 isolate and subject isolates to laboratory analysis (phage typing, selected biochemical tests, exoprotein electrofocussing profiles, gas chromatography) to identify, solely on these criteria, isolates belonging to the same lineage.

4. Form clusters of cases on the basis of the lineage of the V. cholerae isolated. Map the cases in each cluster over time and describe the most probable transmission pathway(s) linking them.

5. Compare different transmission pathways and identify common patterns in time and space characterstics (time between cases, duration of pathway, vector, etc.) by cluster analysis.

6. Attempt to establish functional relationships bewteen movement of cholera along individual pathways and the physical features of the region, especially waterways.

7. Look for changes in these patterns with advancing season and attempt to relate this to seasonal changes in the environment.

C. Methods of Procedure

1. Identification of Cases

The most efficient procedure is probably to get a rectal swab from every person seeking treatment from any of the treatment facilities supported by ICDDR,B in the Matlab area. In addition to obtaining the set of R/S, we will fill out a a form identifying patient name, VTS number (if available), village name and number, bari name, and severity and time of onset of diarrhea.

The rectal swabs will be processed by routine direct plating and enrichment methods and Vibrio cholerae 01 will be identified by colonial morphology, oxidase test, string test and agglutination with group antisera. Each isolate will be stocked in duplicate and sent to the Dacca lab on a weekly or twice-weekly basis.

A detailed physical map of the study area will be prepared showing all rivers and canals and their interconnections at the start of the study period. This will be annotated to show changes in their interconnections as the water level drops. This map will also show the location of each village and, if possible, each identifiable neighborhood so that their relation to water sources can be seen. Neighborhoods will be defined as geographic clusters of households that lead to shared use of the same set of water sources. The relationship between bari and neighborhood will be examined for each village at the start of the study.

Remaining questions: Is this process going to be able to identify most of the V. cholerae 01 associated diarrheas in the population? If not, does using only those cases that come for treatment bias the sample in terms of geographic or social characterstics? If so, how? Are any accurate physical maps of the Matlab area currently on hand or obtainable?

2. Monitoring environmental parameters

Monitoring points should be established at several locations in the Ghumati river to provide reference data on the hydrologic characterst

of the waterways in the Matlab area. The data to be collected at these points will include the depth of the water column and rate of flow and direction.

These measurements can be made with the aid of simple self-registering tidal and flow gauges that utilize spring-driven drums to record changes over 24 hour periods. In addition, information about the times of high and low tide as well as rainfall can be obtained from the Bangladesh government weather service. Temperature, dissolved oxygen can be measured at the site of sampling.

Hydrologic data about the smaller rivers and cannals in the study area can be obtained by monitoring them at weekly intervals. The data collected at these sites can be compared to the data obtained during the same period at the reference points on the Ghumati. By collecting data daily at reference points and weekly at all others, it should be possible to produce a model that will predict the study area's daily hydrologic cycle and, by extrapolation, the hydrology of the smaller rivers and canals on days they were not monitored. If so, we can decide what water connections existed for each day in the study season and how fast water was flowing between villages and the direction of flow at various times during each of these days.

The presence of algae and plankton will be done by collecting water for algae examination and towing nets for 15 minutes for planktons. These will be characterized by special methods described elsewhere.

3. Laboratory Characterization of V. cholerae isolates

The key to this study is our ability to type isolates so precisely that we are able to identify organisms that are in a direct line of descendants for the same ancestor (i.e. lineage). The length of time during which a single lineage needs to be tracked, fortunately, fairly short 3-4 months at most. This improves the chance that major changes in phenotypic characters will not have occurred between the earlies and lates isolation of the same lineage. This will, I hope, translate into a high degree of homogeneity within clusters of isolates.

The laboratory analyses that can be used to provide enough epidemiological markers to support this kind of cluster analysis are:

Phage typing using the typing system that John Lee had established at the VRI at Maidstone.

b. Selected biochemical tests (need to be amenable to rapid batch screening).

1. Fermentation of carbohydrates
2. Antibiotic resistance
3. Sole carbon and energy sources
4. Hydrolytic enzymes

c. Plasmid screen - use minilysates to screen for plasmids on agarose gel.

d. Exo-protein electrofocussing profiles - grow cells under rigidly standardized conditions in dialysis bags filled with buffer and place in flasks of media (casamino acids - yeast extract broth?). At stationary phase, the dialysis culture is centrifuged and filtered to move cells. The culture fluid is then concentrated using MiniCon systems and electrofocussed in agarose gels in the pH range 3-10. Twenty or more samples can be run at one time. The migration and density of each band is then determined. The band patterns from all isolates are subjected to cluster analysis to assign them to specific patterns.

e. Gas liquid chromatography - This is another means of identifying many products simultaneously and identifying patterns that can be evaluated in a manner similar to electrofocussing profiles.

Phage typing and biochemical testing could be done in Dacca. If facilities are available, the plasmid screening, exoprotein analysis and GLC could also be done in Dacca. The latter assays could also be done in Baltimore since all necessary equipment is currently available.

The data that will come from these laboratory analysis will be used to place each isolate in the most probable lineage. This will be determined on the basis of cluster analysis of the phenetic characters found to provide the greatest diffierentiation bewteen isolates.

All isolates will be picked directly from purity plates to prepare permanent frozen stock in BHI+15% glyceral at -70^{C}. These low passage

stocks will provide the source of material for laboratory analyses and, also, permanent reference collection for possible future studies.

4. Analysis of cases and description of transmission pathways

Once all isolates have been assigned to a lineage, cases associated with each lineage will be assumed to describe the pathway of that lineage as it passed through the study area. First, maps will be prepared for each lineage showing location and time of each case so that obvious clusters of cases and transmission pathways can be easily identified.

The next step will be to describe the most probable transmission pathway for each lineage. All cases will be placed in chronological order within each lineage. We will assume that no inter-neighborhood or inter-village transmission could have been involved in cases occurring within 24 hours of one another. We will also assume that any case occurring within 7 days of another case in the same neighborhood has resulted from intra-neighborhood transmission. The cases in each lineage will be grouped to reflect these assumptions. The transmission pathway for each lineage will be described initially by assigning on the basis of temporal sequence, a main line to the series of first cases in each village. Branch lines will then be drawn to reflect intra-and inter-neighborhood transmission from the first case in each village.

5. Comparison of different transmission pathways and identification of common pattern.

Characterstics of case occurrence that can be subjected to cluster analysis include:

a. Duration and total distance covered by a pathway.
b. Number of cases in a pathway.
c. Number of villages involved in one pathway.
d. Time between successive introductions into different villages.
e. Distance between successive villages involved in pathway.
f. Frequence of multiple case outbreaks in same neighborhood compared to single case outbreaks.
g. Number of neighborhoods in village ultimately involved after cholera introduction.

h. Time between each successive neighborhood involvement once cholera is introduced into a village.

i. Overall direction taken by pathways.

6. Examination of functional relationships between transmission pathways and physical features of the region, especially waterways.

If the common patterns observed in intervillage or interneighborhood transmission suggest the involvement of flowing water, this relationship can be examined more closely. One way is simply to evaluate the degree to which cases in a lineage are associated with connecting waterways using cluster analysis. Another approach is to compare the values for transmission parameters predicted from actual isolation patterns (from 5, above) to the water flow characterstics predicted by the hydrologic model described earlier. If cholera is water-borne between villages, there should be substantial areas of agreement. Also, the changing patterns in transmission with advances in the season should be explainable, at least in part, from seasonal changes in hydrology.

7. Identification of changes in transmission patterns with advancing season

Assuming that the previous analysis will show inter-village transmissions to be via the region's waterways and will reveal consistent patterns in the occurrence of cases, these patterns may change as a function of seasonal changes. Such seasonal changes might include decreased flow of water, increased distances between villages as waters recede to their original more winding channels, and substantial tidal effects that diminish net movement along the channel. It is possible that such changes will alter the efficiency of cholera transmission and that this alteration may show up as changes in patterns of transmission.

Additional points: The cluster analyses and modeling proposed in section 2-7 will require a moderately sophisticated computer with statistical packages and graphics capability. These analyses can be done in Dacca if the facilities will be available by early 1982. On the other hand, the analyses can be done at the Johns Hopkins University Computer Center.

D. Significance

Until we know how cholera is transmitted through an endemic region, we will find it extremely difficult to explain seasonality in a satisfactory manner. Without knowing the mechanisms governing the seasonal appearance and disappearance of cholera or governing its spread from village to villages, we lack knowledge that is important in the development and evaluation of optimum environmental interventions. As the experience of the tubewell program in Matlab has demonstrated, the premise that cholera is water-borned, no matter how well-founded, is by itself an insufficient basis on which to plan or evaluate an intervention program. The approach described here offers an opportunity to isolate and analyze portions of the set of ovelapping transmission events that make a cholera epidemic We may, thereby, be able to describe how cholera is transmitted through the region and identify important patterns in this transmission. If so, this may lead us to some understanding of the association between environmental and chemical factors and seasonality and may suggest effective interventions.

E. Facilities

1. Lab Space: We should be able to coordinate most of the bacteriological laboratory work with the routine lab work already on-going. In Dacca we will need space to do the initial characterization of isolates and phage typing. If the other lab analysis are to be run in Dacca (biochemical) tests, GLC, electro-focussing) space and equipment will be needed.

2. Animal Resources: None

3. Logistical Support: Trips to Matlab and speedboat transport in Matlab for a period of about six monts.

4. Specialized Requests: Computer with graphics capability and statistical programs for cluster analysis and other statistical procedures - available in Baltimore.

F. Suggested arrangements and scheduling

The field portion of this study will occupy may through September, 1982. Bill Spira will come to ICDDR,B in May, 1982 to begin this phase of the project and will continue through August.

Imdadul Huq will oversee the project in October to January 1982 and will send the V. cholerae isolates from different parts of the study area to Maidstone for phage typing. If the phage typing proves meaningful in working at a shorter time the transmission pattern, Dr. John Lee will be brough to Dacca to monitor isolates from the study and to train people to continue the work. This will effect in June, 1982. During his stay from May for 6-8 weeks Dr. Spira will finish up the field work with Dr. Huq.

The rest of the schedule depends on where we decide to carry out the rest of the laboratory characterization of the isolates and the statistical analysis. In any case, John Lee would presumably do some work in England to investigate any difficulties or unexpected or ambiguous results in the phage typing of these isolates. Most of the Computer Work will be carried out by Bill Spira in Baltimore.

Papers arising from this study would have as first author: John Lee for those on phage typing, Imdadul Huq or John Lee for those on taxonomy or laboratory characterization, and Bill Spira and Imdadul Huq for papers on aspects of transmission or hydrology.

BUDGET

The only source of funding other than ICDDR,B for this project seems to be the World Health Organization. W.H.O, through the Diarrhoeal Disease Control Program grants, might be prevailed upon to provide support for non-professional salaries and supplies and equipment to carry out the following laboratory analyses: biochemical tests, plasmid screening, electrofocussing profiles of exo-protein, and GLC of cells.

The major costs that would be left to ICDDR,B include:

1. Travel
   One round trip from Baltimore to Dacca for Bill Spira
   One round trip from London to Dacca for John Lee.

2. Costs of cholera surveillance, hydrological monitoring, initial characterization and phage typing of isolates, and preparation and shipping of stock cultures.

   The costs of analysis: Computer time, programmers and analysts fees, will be borne by Dr. Spira (from grants in Baltimore).

08/03/81 - Revised Schedule of Work for Matlab Intervillage Cholera Transmission Study.

Budget considerations and questions about the usefulness of V. cholerae phage typing require some changes in the schedule of work originally proposed. The revised plan will be to: 1) carry out a modest surveillance of cholera cases; 2) follow this with lab work to evaluate the Maidstone phage typing system and to assess other methods of identifying epidemiological markers, and 3) carry out preliminary pattern analysis of intervillage cholera transmission on the data thus far. If these efforts are successful, a more definitive prospective study of transmission would be carried out during the 1982 cholera season.

Dr. Colwell is arranging for satellite photographs of the study area at 18-day intervals throughout the proposed period of surveillance this fall. This being the case, it would be highly desirable to gather as much data as is feasible. Fortunately, it seems that the most crucial aspect of the original proposal, the Matlab hospital and OPD cholera surveillance, is still intact. I propose that we carry out, in addition, about 10 one-day surveillance visits to neighborhoods of cholera index cases to obtain clusters of V. cholerae isolates. The purpose of these clusters would be to assist us in evaluating the usefulness of phage typing and other epidemiologic markers. It would be expected, I think, that all isolates within one of these clusters should be the same strain and, hence, should differ little in their epidemiologic markers. If there are significant differences, this casts doubt on the stability of the markers or it suggests multiple strain involvement in neighborhood outbreaks. Either alternative leads to intriguing questions that might serve as the basis for future studies. Assuming, however, that significant differences aren't found in these clusters, they provide a benchmark for assessing isolates from other areas or times in the study.

It is critical that all isolates be stocked forzen at -60C in BHI-glycerol immediately from purity plates. These purity plate cultures can then be tested using the routine confirmatory tests available at ICDD,B. On-going plasmid screening of isolates as they become available in Dacca would be very desirable if the manpower will be available to do it.

Phage-typing of a randomly selected sample of hospital and OPD isolates and of all index case/neighborhood clusters will be carried out by Dr. Lee in Maidstone during the fall and winter. The results of phage typing will, thus, be available, by February or March, 1982. If these results confirm the usefulness of Dr. Lee's system, he should be scheduled to come to Dacca in the spring to train several technicians and to set up the necessary equipment and stocks. This will allow the lab to become familiar with this assay over several months before the next season's work is scheduled to begin.

In February, 1982, I would like to have Qazi Shafi Ahmed come to my lab in Baltimore for a four month training period during which time we would evaluate the usefulness of exoprotein profiles and GLC patterns as epidemiologic markers. We would use the same study set that John Lee uses and, I hope, that had been already screened for plasmids in Dacca. The results of this work would be known by June, 1982, in time to use the assays during the next season's study if indicated. I have sufficient funds to support Qazi Shafi during his stay at the Johns Hopkins University if the ICDDR,B is willing to assume his travel expenses.

Analysis of the data collected thus far, particularly the proposed pattern analysis of transmission using the epidemiologic markers being evaluated, will be carried out in Baltimore. The Division of Geographic Medicine has recently established an in-house microcomputer facility that will be further upgraded during the coming year. This system will have graphics capability and access,

via MODEM links, to interactive statistical routines needed to identify characteristic features of cholera transmission. Perhaps, this might suggest mechanisms of the seasonal spread of colera through this area.

The analysis must be preliminary, however, since we are identifying only those infections leading to hospital or OPD visits. The biases this procedure introduces are obvious. The excercise, however, may reveal patterns that can be further tested in a follow-up prospective study in the Fall, 1982. Such a study will not be practicable unless the number of mechanisms to be examined can be reduced. Assuming we are successful in identifying usable epidemiologic markers and in revealing characteristic trajectories in intervillage cholera transmission, we can focus on one or more of the following mechanisms:

1. Transmission associated with waterways.
   a. Mechanical transmission by flowing water (in water columns, associated with plants, zooplankton, etc.)
   b. Transmission of human infectives along water routes (boatmen, fishermen).
   c. Transmission by animal infectives along water routes (fish, gulls).
   d. Transmission by contaminated foodstuffs transported via water.
   e. Multifocal diffusion-like propagation from seasonal blooms of autochtonous aquatic toxigenic V. cholerae 01 in the region's waterways.
2. Transmission associates with overland movement.
   a. Diffusion-like transmission of cholera by human infectives moving from village to village.
   b. Transmission by animal infectives (chickens, wild birds, rodents, etc.).

c. Transmission by flies.

d. Transmission by food.

This list is not meant to be exhaustive. It does, however, cover the mechanisms most likely (on current information) to be the important ones. It suggests the extreme size of any prospective study begun without preliminary weeding of possibilities. Brief consideration of the list also suggests that different mechanisms should lead to identifiable differences in propagation patterns. Thus, one of the most effective bases for weeding out possibilities might be the identification of characteristic patterns in cholera propagation in the Matlab area from our preliminary data.

I am holding back the WHO proposal discussed earlier this year until Spring, 1982 to give us a chance to sort out the future of this study. My current feeling is that the concept of the WHO proposal should be extensively reworked. It now encompases only the laboratory work which was to have followed a major surveillance effort this Fall. Given the changed circumstances and schedule, I think it would be appropriate to request substantial support for the field work in Fall 1982 as well as the laboratory analyses. I would also very much like to include additional studies for the following two years on characteristics of cholera transmission during the smaller dry season peak (with such dramatic differences in environment one might expect equally dramatic differences in transmission patterns) and a comparative study on the epidemiology of toxigenic and bioactive non -1 V. cholerae diarrhea with that of cholera. This is something that I think must certainly be discussed and, I hope, settled during my stay at ICDDR,B this Fall.

The budget I submitted as amended by the Disease Transmission Working Group for the portion of the study to be done this Fall is acceptable with the provision that funds for travel by Dr. Lee and Q. S. Admed should be

made available in Spring 1982. Dr. Lee's travel to Dacca will, of course, be contingent on the results of the phage-typing of the study set to be collected. Additional amounts for supplies and equipment will also be needed for this work. I understand from Dr. Huq that this budget can be agreed upon later this year but it can be formulated while I'm in Dacca.

SECTION III - BUDGET

A. DETAILED BUDGET

1. PERSONNEL SERVICES

	Effort	Annual Salary	Project requirement Taka	Project requirement Dollar
Dr. W.M. Spira	-	-	-	-
Dr. M.I. Huq	15%	$ 24,000	-	3,600
Dr. John V. Lee	-	-	-	-
Mr. Q. Shafi Ahmed	25%	Tk. 65,800	16,470	-
Mr. M.A. Salek	50%	Tk. 56,900	28,450	-
Mr. M. Ansaruzzaman	100%	Tk. 36,995	36,995	-
Mr. Belayet Hossain	25%	Tk. 36,612	9,153	-
Field Worker (to be named)	100%	Tk. 24,000	24,000	-
Md. Ismail	50%	Tk. 16,534	8,267	-

2. SUPPLIES AND MATERIALS

a.	Cost for testing water including biochemical confirmation of *V. cholerae*.	2,000 @ Tk. 20.00	40,000	-
b.	Stock vials	1,000 @ Tk. 4.00	4,000	-
c.	Maping, Supplies etc.		4,000	-
d.	Antibiotic sens. phage typing and other tests	1,000 @ Tk. 10.00		
e.	Office supplies		3,000	-

3. EQUIPMENT

a.	Lidwell pattern phage typing machine	-	650
b.	Flowmeter	-	400

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	Taka	Dollar
4. HOSPITALIZATION - None		
5. OUTPATIENT CARE - None		
6. ICDDR,B TRANSPORT		
Speedboat in Matlab 150 miles/100	15,000	-
Land transport 1000 miles/4.00		
7. TRAVEL & TRANSPORTATION OF PERSONNEL		
Round trip, air fare for Dr. Spira Baltimore/Dacca/Baltimore	-	1,400
Round trip air fare for Dr. Lee London/Dacca/London	-	1,000
8. TRANSPORTATION OF THINGS		
Supplies Equipments, and Frozen specimens	-	300
Shipment of supplies to & from Matlab	1,500	
9. RENT		
Guest House charge for Dr. Spira & Dr. Lee Total 80 days @ $ 30.00	-	2,400
10. PRINTING AND REPRODUCTION		
Xerox	2,000	-
Other	1,000	-
Publication cost	-	200
11. CONTRACTUAL AND OTHER SERVICES		
Computer work at Dacca		
Programmer	2,000	-

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	Taka	Dollar
Key punching	2,000	-
Computer time (if needed in Dacca)	10,000	-

Computer time in Baltimore will be borne by grants of Dr. Spira.

B. BUDGET SUMMARY

		TAKA		DOLLAR
1.	Personnel Services	123,335.00		3,600.00
2.	Supplies & Materials	62,000.00		-
3.	Equipment	-		1,050.00
4.	Hospitalization	-		-
5.	Outpatient Care	-		-
6.	ICDDR,B Transport	19,000.00		-
7.	Travel			2,400.00
8.	Transportation of thing	1,500.00		300.00
9.	Rent/Communication	-		2,400.00
10.	Printing	3,000.00		200.00
11.	Contractual Services	14,000.00		-
12.	Construction	-		-
	Total -	231,835.00	US$	9,950.00
	@ 18.00/US$	12,879.00		
	Grand Total US$	22,829.00		