REVIEW BOARD ON THE USE OF HUMAN VOLUNTEERS

CRL

63

Principal Investigator W. SPIRA J. BRISCOE Trainee investigator(if any) _____

Application No 77-032 Supporting Agency(if Non-CRL) _____

Title of study Fecal coliforms and fecal streptococci as indicators of fecal contamination of water sources in rural Bangladesh.

Project status:
(L) 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 N/A)

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 possibly 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 N/A
   c) Physical risks Yes N/A
   d) Sensitive questions Yes N/A
   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 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
7. Check documents being submitted herewith to Committee:
   ___ Umbrella proposal - Initially submit an overview(all other requirements will be submitted with individual studies).
   L Protocol (Required)
   L Abstract summary (Required)
   L 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 sensitive or which would constitute 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 Board for review.

We agree to obtain approval of the Review Board on Use of Human Volunteers for any changes involving the rights and welfare of subjects before making such change.

Principal Investigator

Trainee

Please return 2 copies of entire protocol to Chairman, Review Board on Use of Human Subjects.

Received 16/11/77
77-032

Review Board on the Use of Human Volunteers

ABSTRACT SUMMARY

Fecal coliforms and fecal streptococci as indicators of fecal contamination of water sources in rural Bangladesh

1. Persons asked in this study to supply stool samples or to provide information will be taken from other on-going studies in CRL. This study places no additional burden on these persons.

2. There is no risk involved in this study.

3. No risks are involved.

4. Data will be reported without reference to the name of participants.

5. No risks of any sort are involved. Persons will be told of the nature of the study and of their right to refuse to participate.

6. The interview will take place in the homes of persons in the Fatepur study area at monthly intervals. Each interview should take no more than five minutes.

7. There are no benefits to the individual. Public health may ultimately be improved through interventions developed using the monitoring tools defined in this study.

8. This activity requires the use of no records, organs, tissues, body fluids fetus or abortus.

Fecal coliforms and fecal streptococci as indicators of fecal contamination of water sources in rural Bangladesh

STATEMENT TO BE READ TO RESPONDENTS OF QUESTIONAIRE

We want to know what places you used for defecation in the past 24 hours. This will help us in a study of the best way to test water quality. This test can then be used to show how much improvement in water quality results from different sanitation methods.

SECTION I - RESEARCH PROTOCOL

1) Title: Fecal coliforms and fecal streptococci as indicators of fecal contamination of water sources in rural Bangladesh

2) Principal Investigators: William Spira, John Briscoe

3) Starting Date: 15 November 1977

4) Completion Date: 30 July 1978

5) Total Direct Cost: $ 11,877

6) Abstract Summary:

The concentration and composition of fecal coliforms (FC) and fecal streptococci (FS) in the feces of humans and animals will be determined. The viability of FC and FS under a variety of circumstances and other factors which are likely to affect the presence of these organisms will be evaluated independently. This will be sufficient to validate the use of these measures as indices of fecal contamination in this geographical area. A surveillence of selected water sites will be carried out to determine FC and FS concentration and monitor the environmental and behavioral factors acting on them. A mathematical model will be developed to describe the effects of various factors on the presence of FC and FS. This model will be calibrated using the study data. It will then be used to evaluate the utility of FC and FS as indicators of extent and source of fecal pollution-limiting conditions which must be met, factors which affect the interpretation of results, and the confidence which can be placed in them when extensive verification is not possible. The model may also be used to evaluate the impact of various conditions on the extent of fecal pollution in a natural water source. This may lead to some insight into seasonal variations in fecal pollution and, perhaps, the incidence of water-born fecal-oral pathogens.

7) Reviews:

a) Research Involving Human Subjects: _____

b) Research Committee: _____

c) Director: _____

d) BMRC: _____

e) Controller/Administrator: _____

SECTION II - RESEARCH PLAN

A. INTRODUCTION

1. Objective: The overall objectives of this research are to evaluate the usefulness of fecal coliform (FC) and fecal streptococci (FS) counts and the FC:FS ratio as indicators of the extent and source of fecal contamination in water, and to provide, through simulation models, a coherent description of those factors which affect the presence of fecal pollution in water sources.

2. Background: The evaluation of water quality depends, to a great exten on an assessment of how liable a water body is to be contaminated by pathogens of fecal origin. Since it is usually difficult to isolate pathogenic microorganisms directly, organisms unique to, but common i , feces are monitored instead as an index of fecal pollution. Such measurements are important for several reasons. They provide a means of determining whether a particular water source is safe to use. They also provide a means of investigating mechanisms which control the transfer of fecal matter into water sources and which determine the concentration of contaminants under varied environmental circumstances. Ideally, a measurement of indicator organisms should yield an estimate of the extent of the pollution as well as differentiate between pollution by man and by other animals.

Although total coliform counts are still used to assess pollution, indicator organisms which are more directly associated with the

intestinal tract of warm-blooded animals are preferable. These include fecal coliforms (FC: in practice this is synonymous with Escherichia coli) and fecal streptococci (FS) (Geldreich, 1966; Slanetz and Bartley, 1964). Since these organisms are normally associated only with the gut of man and other animals, and tend to die off quickly once they are deposited in water (McFeters, et al., 1974), their concentration in water serves as an index of recent fecal contamination and of the potential presence of pathogens. Media and methods for the recovery and differentiation of these indicator organisms have been the subject of intensive study. A variety of quantitative methods, including most-probable-number (MPN) and membrane filtration techniques, have been developed and standardized (APHA, 1971).

Routine determination of FC depends on the ability of this group to grow at 44.5C and ferment lactose. An MPN procedure utilizing lactose and EC broths and a membrane filtration technique using m-FC broth as a growth medium (APHA, 1971) are the two most commonly employed methods The membrane filtration procedure appears to be of greater utility for field studies (Feacham, 1974) though the MPN technique is somewhat better for recovery (Bissonette et al., 1977). FS are usually determined by a membrane filtration technique at this time, using either m-enterococcus agar (Slanetz and Bartley, 1957) or KF streptococcal agar (Kenner et al., 1961). KF streptococcal agar is reported to be less selective against fecal streptococci from non-human sources than m-enterococcus agar, though some investigators have experienced reduced recovery of S. bovis on both (Switzer and Evans, 1974). Others, however, have not

noted this phenomenon (Brodsky and Schieman, 1967). On balance, KF streptoccal agar appears to be the medium of choice for determining FS when pollution from non-human sources is anticipated.

A problem with all membrane filtration techniques has been a dramatic variation in recovery with filters of different types and from different manufacturers (Lin, 1976; Green et al., 1975). This appears to be due primarily to differences in the amount of contact between media passing through the filter and the cells embedded on it. This is, in turn, a function of pore character (Sladek et al., 1975). At present, Gelman and Millipore type HC filters seem to give the best recovery rates (Green et al., 1975).

A problem which has been only infrequently appreciated and poorly evaluated is the failure of FC and FS assays to count sublethally injured cells. The phenomenon of sublethal injury and the failure of selective media to detect cells so affected has long been known to occur in foods subjected to stress situations such as freezing, drying, or heating. The same phenomenon has been demonstrated in E. coli exposed in situ to natural waters (Bissonnette et al., 1975). These injured cells were capable of recovery if maintained in a rich medium for several hours before being plated on selective media. The extent to which injury can affect FC and FS counts in highly polluted waters is unknown, though it is likely that the high nutrient concentration would be protective. Bissonnette et al. (1977) have shown that a 2-hour incubation of membrane filters at ambient temperature on supplemented trypticase soy agar

before exposing them to selective media substantially enhanced the recovery of E. coli from pure culture and of fecal coliforms from raw sewage.

The ratio of FC:FS has been proposed as a method for determining whether pollution is from a human source or an animal source (Geldreich, 1966). Table 1 (taken from Geldreich) shows how this is possible. The concentration of FS in animal feces is much greater than that of FC. In humans, the ratio is reversed. Thus, a high FC:FS ratio would indicate human pollution; a low ratio, pollution from an animal source. In practice, a ratio of 4.0 or greater is considered an unequivocal indication of human pollution; a ratio of 0.7 or less of animal.

A major weakness in this method is that it properly applies only to water which has been very recently contanimated with fresh fecal matter, since FC and FS die off at different rates, thus altering the FC:FS ratio (McFeters et al., 1974). Since the age of pollution is difficult to judge, the FC:FS ratio would appear to be an unreliable indicator of pollution source. A modification of this procedure suggested by Feacham (1975), however, promises to overcome this difficulty and, indeed, provide an even better indication of pollution source. Feacham has noted that enterococci (S. faecalis and S. faecium), which dominate the FS from human feces, survive better than FC which in turn survive better than the predominant FS in animal feces (S. bovis and S. equinus). He proposes that a series of measurements of FC and FS concentration be taken through time and the FC:FS ratios plotted. The slope would indicate

Table 1: Estimated per capita contribution of indicator microorganisms from some animals.

Animal	Avg wt of Feces/24 hr wet wt, g	Avg density per gram of feces (million)		Avg contribution per capita per 24 hr (million)		Ratio FC:FS
		FC	FS	FC	FS	
Man	150	13.0	3.0	2,000	450	4.4
Duck	336	33.0	54.0	11,000	18,000	0.6
Sheep	1,130	16.0	38.0	18,000	43,000	0.4
Chicken	182	1.3	3.4	240	6,200	0.4
Cow	23,600	0.23	1.3	5,400	31,000	0.2
Turkey	448	0.29	2.8	130	1,300	0.1
Pig	2,700	3.3	84.0	8,900	230,000	0.04

whether the source of the pollution were human (falling slope) or animal (rising slope). Data from studies by McFeters et al. (1975) and Feacham (1974) could be interpreted to support this proposal.

The validity of this approach and its usefulness, as well as the usefulness of enteric indicator bacteria in general, is likely to be strongly related to the geographical area studied and the nature of the pollution sources involved. Evison and James, (1973), for example, found that FC were able to survive for long periods of time in tropical waters exposed to a high ambient temperature. This would obviously affect their utility as an index of recent fecal contamination. The possibility that, in very hot areas, the FC group might contain a large number of non-fecal saprophytes should also be considered, though White et al., (1972) found in East Africa that 90% of all FC were, in fact, E. coli type I. In another vein, McFeters et al., (1974) demonstrated that the FS in elk feces declined at a significantly slower rate than the FS in the feces of other animals. The rate was very close to that of FC, so there was virtually no change in FC:FS ratio with time.

It is evident that the use of indicator bacteria and the FC:FS ratio as a means of assessing fecal pollution must be approached carefully. A proper interpretation of these measurements will require some information on the fecal flora of humans and animals in the study area -- concentration of FC and FS, composition of streptoccal flora -- as well as estimates of viability decay rates under the range of environmental conditions to which the indicator organisms are exposed. Feacham's (1974) study of

FC and FS in New Guinea Highland streams depended on data from the United States on the microflora of human and animal feces for his analysis. The appropriateness of these assumptions is, as he admits, not known. Using this data, however, he showed that overland rainfall run-off into streams was a mechanism of major importance in transferring fecal material. He was then able to relate peaks in FS concentration to the washing of pig feces deposited along the banks into streams. Thus, he was able to gain some understanding of the significance of the changes he observed in the concentration of indicator organisms with changing environmental conditions.

In general, most of the work relating to indicator organisms in natural waters has been focussed primarily on coliform and fecal coliform survival and growth rates in the natural environment (Hendri 1972; McFeters and Stuart, 1972). Very few attempts have been made to relate microbial concentration to environmental factors, probably because of the extreme complexity of the problem. Brasfield (1972) made an effort to develop multiple regression models for predicting coliform populations in a stream as a function of various environmental factors. A comparative analysis of several deterministic and probabilistic models for coliform organisms has also been reported (Mahloch, 1974). A major difficulty with these efforts is that the general applicability of the models is poorly defined. Mahloch's findings show clearly that models and constants developed under one set of conditions cannot justifiably be used to describe a different set. When properly limited, however, and accompanied by extensive verification, such

modeling efforts appear to be useful analytic and didactic tools for describing fecal contamination of natural waters.

3. Rationale: Careful observation and measurement of FC and FS in wat sources and of the factors which are likely to influence their pres. . . will yield sufficient data to evaluate the utility of indicator organisms as indices of fecal pollution. Analysis carried out through the use of formal models will also focus attention on the influence of specific factors on FC and FS presence and on conditions which affect the manner in which their presence ought to be interpreted; and will permit us to draw firm conclusions on the utility of indicator organisms and the variability of fecal contamination of water sources under different sets of conditions.

B. SPECIFIC AIMS

1. Estimate the range of values for each of the variables likely to have an impact on the number of FC and FS in water. These include

   a. Concentrations of FC and FS in freshly passed animal and human f and their change with time as a function of environmental condit o .

   b. The change with time of FC and FS concentration in water freshly contaminated with human or animal feces as a function of environmen conditions.

   c. The number of FC and FS added to water during bathing, clothes washing, or ablution.

   d. The number of FC and FS transferred by overland run-off from stool located in the catchment area to a body of water as a functi of propinquity and rainfall.

e. Rate of dispersion of FC and FS in a body of water as a function of climatic conditions and type of water body.

f. The reduction of mass of stool deposited in the catchment area of a water body with time.

2. Monitor selected sites in different water bodies to obtain data on: FC and FS concentrations; people's defecation, ablution, bathing, and clothes washing patterns; animals defecation and bathing patterns; an environmental parameters (air/water temperature; pH and dissolved oxygen tension in water; sunlight; rainfall, wind velocity and movement of water-flow or tidal action).

3. Develop a model to describe the effects of various factors on the presence of FC and FS in a water body and calibrate it using the study data. Use this model to:

a. evaluate the utility of FC/FS ratios as an indicator of the source of fecal pollution and of FC and FS concentrations as an index of the level of recent fecal contamination under different sets of conditions.

b. evaluate the effect of behavioral, physical or biological factors on the level of fecal pollution of water sources and consider how this may relate to the characteristics of water-borne diarrheal disease transmission in Bangladesh.

METHODS OF PROCEDURE

Quantitation of FC and FS. Laboratory procedures for the determination FC and FS will be highly standardized. The procedure developed for

processing samples from the longitudinal field study will be followed for all samples, including those taken during laboratory experiments to ensure consistency. The same lot of m-FC broth and KF Streptoccal Agar will be used throughout. Differences between lots of membrane filters not significant determinants of fecal indicator recovery rates (Green et al., 1975) so no special effort to obtain a single lot will be made, though the same type and manufacturer (Gelman) will be used throughout.

Samples of water or feces will be membrane filtered immediately upon collection. All dilutions will be made in gelatine-phosphate buffer, pH 7.0 (Bissonnette et al., 1975). Water sample volumes will be brought to 30 ml with buffer in preparation for filtering. If necessary, serial dilutions will be made by adding 1.0 ml to 2.16 ml of buffer in 10 ml screw cap tubes. Each dilution will be mixed thoroughly by shaking the tube vigorously through an arc of 2 feet at least 20 times before preparing the next dilution. One ml will be pipetted into the filtration cup then 30 ml of buffer will be added. This procedure will give a final dilution of 0.316x the preceeding tube. Fecal samples will first be mixed with buffer in the ratio of 5g:45ml in a 4 oz jar containing glass beads. The fecal matter will be broken up thoroughly and suspended as finely as possible by vigorious shaking through an arc of two feet at least 40 times. Suspended particles will be allowed to settle for one minute then a 10 ml aliquot will be removed from the supernatnat fluid and serially diluted as described above.

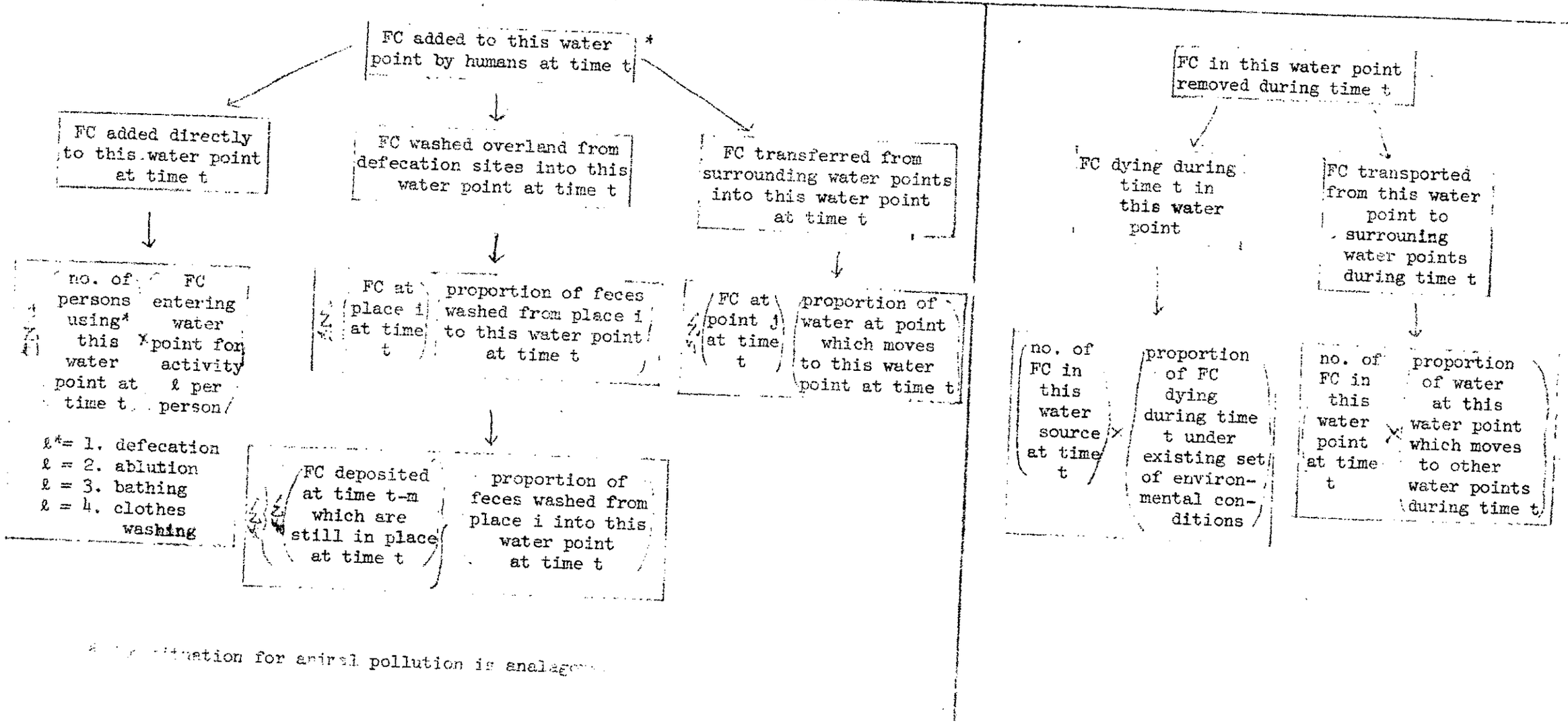
Membrane filtration will be done by the standard technique described in APHA (1971). Filters for both FC and FS analysis will be transferred to pre-chilled plates of TSY (trypicase soy agar plus 0.1% yeast extract plus 0.5% glucose). These will be held in an ice chest at no greater than 8° C until they can be returned to the laboratory. Plates will be maintained in this manner for no less than two hours and no longer than 12 hours. Plates will then be warmed to 25° C and held for two hours. Filters for FC analysis will then be transferred to plates containing filter pads soaked with m-FC broth (BBL) and incubated at 44.5 ±0.2° C in a covered water bath (plates will be sealed in plastic bags). Blue colonies formed after 24 hours incubation will be counted as fecal coliforms. Filters for FS analysis will be placed on plates of KF Streptococcal agar (BBL; prepared by boiling rather than autoclaving) and incubated at 35° C for 48 hours. Red to pink colonies will be counted as fecal streptococci. APHA (1971) procedures will be followed throughout this process.

FC colonies will be confirmed using KIA, MIU, MR-VP and Simmons citrate agar. FS colonies will be characterized by Gram stain, catalase reaction, growth at 10 and 45° C, growth on bile-esculin agar (Facklam and Moody, 1970), tolerance to 60° C for 30 minutes and by the ability to ferment lactose. These tests will be carried out only when it is necessary to characterize isolates to the species level.

Estimation of variables which affect FC and FS presence. The rationale underlying the model for FC (or for FS) -- the structure of which is defined in the following section is presented in Fig. 1. In order

Fig. 1: Factors affecting presence of FC in a particular water point at a given time.

FC in water at time t = FC in water at time t-1 + RC added at time t from humans + FC added at time t from animals - FC removed at time t



* situation for animal pollution is analagous

develop and evaluate this model as well as to validate the use of FC and FS counts in this geographical area it will be necessary to obtain independent estimates of variables which are to be included. These will be obtained in the following manner:

1. Concentration of FC and FS in fresh stool and change with time.

Freshly passed stool from human volunteers will be collected from persons involved in other CRL field work such as parasite surveillence or nutritional studies as well as from our study area. When possible, FC and FS determination will be made on stools collected routinely as part of other studies. All volunteers donating stool will be read an explanatory statement and asked to give verbal consent. Stool for FC/FS analysis will be stored in an ice chest at $\leq 8^{\circ}C$ for more than four hours before being returned to the laboratory and processed. Stools will be classified with regard to physical character (formed, soft or liquid), age of donor (0-10 yrs, over 10 years), and sex of donor. Three stool samples for each classification set, or 24 in all, will be collected and processed in November, January, April, and July. In addition, two of the stool samples will be selected each period to evaluate the change in bacterial concentration with time. Portions of each stool sample will be exposed to temperatures of $15^{\circ}C$, $25^{\circ}C$ or $35^{\circ}C$ and to water saturated or drying conditions. The latter will be achieved by holding stool in the presence of Drierite at a ratio of 1:1 (w/w). Samples will be collected and processed at 0, 24, 48 and 72 hours.

Five freshly passed stool specimens each will be collected from cows, pigs, goats, and chickens in the same month that human samples are collected and treated in the same manner. In the same way, two samples from each animal will be used to evaluate the change in bacterial concentrations with time.

2. Change in FC and FS concentration in water freshly contaminated with human or animal feces. Untreated water samples will be contaminated with fecal matter in the ratio of 100 ml of water per gram of feces. If volumes of 1 liter of less are used this will be accomplished by adding fecal matter directly, shaking for one minute, then allowing solid material to settle and decanting. For larger volumes, a 1 liter aliquot will be exposed by shaking then thoroughly mixed back. FC and FS dererminations will be made immediately before and after contamination and at 24 hour (or shorter in the case of animal feces) intervals thereafter. The following conditions will be tested:

a. Variation between animal types - water from two sources will be contaminated with feces from human, cow, pig, goat or chicken; and held at 25° C, pH 7.5 (adjusted) away from sunlight. Three repetitions for each fecal type.

b. Variation between water sources - water will be collected from 20 tank and river sites and contaminated with human or bovine feces; and held at 25° C, away from sunlight. DOT, pH, total viable heterotrophic microbiol population, and phyto- and zooplankton concentration (microscopic examination) will be determined. Each source run in duplicate.

c. Variation due to physio-chemical factors - water from one relatively non-polluted source will be adjusted to the following conditions and contaminated with human or bovine feces: pH 6.0, 7.5, 9.0; sunlight-exposed, not exposed (this will also affect the DOT as a result of photosynthetic activity); and temperature- 15°C, 25°C, 35°C. Each set of conditions will be run in duplicate. Conditions associated with greatest change in FC/FS survival will be tested with pig, goat and chicken feces as well.

3. The number of FC and FS added to water during bathing, clothes washing and ablution. An estimate of these parameters may be adequately obtained by having volunteers carry out these activities in a tub of water. Pre- and post-activity measurements of FC and FS would be made. Ideally, a volunteer would provide a stool sample, carry out his ablution then bathe so that the proportion of FC/FS transferred by each activity could be estimated. Ten male volunteers will be sought for this purpose. It is hoped that sufficient privacy can be ensured so that adults as well as children will participate. An explanatory statement will be read to each volunteer and verbal authorization obtained.

4. The number of FC and FS transferred by overland run-off from feces located in a catchment area to a water body. The variable to be measured is the proportion of FC/FS located in various zones in the catchment area which will be washed into the water body under various rainfall conditions. The actual amount of fecal matter in each zone can be determined by on-site observation. Sodium

fluoroscein will be used as a marker for determining proportional transfer in run-off water. The dye will be incorporated in 200 g dough "pancakes" made from flour, salt and water at a level to be determined empirically. The dough will be standardized to a consistency approximating that of softly formed stool and the "pancakes" will be stored in sealed plastic bags at 4° C to retain the moisture content. These dye-pancakes will be used to simulate for stool deposited around a water source in the following manner.

A tank of fairly large size (i.e. 30 x 30 M or greater) isolated from tidal effects will be selected and the contour of the surrounding land for a distance of 40 M will be mapped. The placement of existing run-off tracks will also be noted. Study zones will be established at shoreline, 1, 5, 10, 20, and 40 M from shoreline and points along each zone line will be tested and time and placement sites will be selected so that the run-off from each can be assessed uniquely. Pancakes will be placed at the start of rainfall. Volume of rainfall will be determined at 30 minute intervals. The dispersal of the dye from each placement site will be watched at 30 minute intervals as well. Run-off water containing as little as 0.1 ppm fluorescein should be visible under these conditions. Water samples will be taken at each point in the water body at which run-off from a placement site is likely to enter. Samples will be collected at 1 hour, two hours, and four hours after rainfall begins and one hour after rainfall ceases. Transects will be established along the shore in either direction from an entry point and directly out into the tank.

Samples will be collected at 1 M intervals for 5 M along each traced line at the sampling times given above. Samples will be taken from surface and bottom using a pole and stoppered bottles. Visible run-off may not be detectable from some distant placement sites. In these cases, a point in the water body directly opposite the placement site will be sampled at 24 and 48 hours after placement it will be assumed that neither overland run-off nor percolatinn through the ground is likely to be a significant transfer factor.

The concentration of fluorescein in a water sample will be determined by titration to a color endpoint compared to a 10 ppb solution of fluorescein. Determination will be made in 20 cm quartz glass tubes under black light illumination. The concentration of fluorescein in the samples taken at run-off entry points and along transects will be determined to calculate the proportion of fluorescein transfered from the placement site up to the time the samples were taken. This will be used as an estimate of the FC/FS transfer rate for a given intensity of rainfall and an estimate for each zone line will be calculated.

Estimates will be obtained for both light and heavy rainfall conditions.

5. Rate of dispersion of FC and FS in a body of water. Fluorescein dye will again be used as a marker. A solution containing 1 g of Na fluorescein in 100 ml of water will be added at various water points and its dispersion will be checked by sampling along four transects (at 90° angles from one another) emanating from the origin.

Samples will be taken from the surface and bottom at 1 M intervals along each transect for a distance of 5 M. Samples will be collec- 1, 2, 4, and 8 hours after dye is added. Points to test will include:

a. Unused tank point -- no wind, rain, tidal action
b. Unused tank point -- tidal action only
d. Unused tank point -- wind action only
d. Unused tank point -- heavy rainfall
e. Canal and river sites -- low flow rates
f. Canal and river sites -- high flow rates

Water flow rates, wind speed and rainfall will be monitored during the period when samples are being collected.

6. Reduction in mass of stool deposited on land with time. This variable will be estimated from daily observation made on isolated identified stool. Most of the reduction in human stool mass will be the result of dung beetles and its rate can be determined by estimating the mass of stool remaining each day for a five-day period. For animal stool (almost entirely bovine) deposited in the catchment area, observation will also include the proportion removed for use as fuel at different times of year.

Monitoring selected natural water bodies. A study area will be established at Fatepur (V-6), which is already being observed for water use and sanitation practices by one of the investigators (J.B.). Two tanks and two canal areas will be selected for study. Each water source

will be mapped and location of ghats, latrines or run-off tracks from adjacent human or animal defecation sites will be noted. The contour of the land within 50 M will also be noted. Sampling sites will be selected in each water body to include:

1. a heavily used ghat (bathing and clothes washing)
2. an active latrine site
3. a site subject to heavy run-off during periods of rainfall
4. center of water source

Sampling and observation will be carried out at monthly intervals beginning in November and ending in July. Each site will be sampled for a period of 24 hours beginning at 0600. The observation period will start 24 hours before the sampling period and end with it.

The sites of all persons who defecate in the catchment area of each water body during the observation period will be determined. Direct observation would be difficult given the variety of defecation sites and the fact that much defecation takes place before or at dawn. In an intensive anthropological study of defecation patterns in West Bengal, Kochar (1975) found that responses to questionairs administered carefully by people known to the villagers were reliable. We will follow this procedure. A local man and five women known to the population will be used to obtain the desired information for each person in the 60 families in the study area. The information will include time and place of defecation and place where ablution was carried out. In addition, the mass of fecal matter in place around each water body at

the start of the observation and sampling periods will be determined by direct observation.

Data on people's bathing and clothes-washing patterns will also be collected through interviews conducted with that for defecation patterns. Since the bathing and clothes-washing sites for each family are known (J.B.'s water study) and since checking of these data by direct observation is not a difficult task, bathing and clothes-washing practices during the sampling period at the selected sites will be observed.

Data on animal defecation and bathing patterns are being collected bi-weekly for 50% of the families in the study population under another protocol (J.B.'s organic materials study). In addition, the cattle owned by four families will be watched throughout the observation period and the quantity of dung produced, the place of defecation and the place of deposition of collected dung recorded. These observations will be done by boys from the study area under close supervision. Bathing of animals at any of the sampling sites during the observation period will also be recorded by persons keeping these sites under observation.

Wind velocity, amount of rainfall, cloud cover and air temperature will be determined at four hour intervals thfoughout the observation period using standard meteriological procedures. The pH, D.O.T. and temperature of water in the top 20 cm of the water column will be determined at each sampling site at the times samples are collected. The water flow rate will also be determined at these times. In addition, the times of high and low tide will be recorded.

Water samples will be collected, using a pole and stoppered bottles, from just below the surface. Samples will be collected from two points within each sampling site. Sampling times, will be established at 0600 and at six hour intervals thereafter including a sample taken 24 hours later. Samples will be processed for FC and FS concentration. In addition, selected samples will be held at 25° C and checked at 24 and 48 hours to determine the change in FC:FS ratio with time.

Model for FC and FS in natural water bodies. The method which will be used for analyzing the data gathered in this study will center on a "materials-balance model" of FC and FS in the water bodies. The rationale for this model has already been presented in Fig. 1. This diagram can now be transformed into mathematical form to provide a simple model (for a closed system) of the process we are trying to understand. The form of the model for FC and FS are identical so that of FC only will be presented here. The terms of this model are defined as follows:

$FC_{J_j,t}$ = total fecal coliforms (FC in source J_j on day t.

$V_{J_j,t}$ = chemical, physical and bacteriological qualities of water body J_j on day t.

$\delta(V_{J_j,t})$ = proportion of organisms dying on day t at water point J_j

$XH_{J_j,t}$ = no. animals bathing at water point J_j on day t.

$XA_{J_j,t}$ = no. animals bathing at water point J_j on day t.

FCHB = no. FC's transferred to water during bathing per person.

FCAB = no. FC's transferred to water during bathing per animal per day.

$YH_{J_j,t}$ = no. people defecating directly into point J_j on day t

$YA_{J_j,t}$ = no. animals defecating directly into point J_j on day t

FCHD = no. FC's passed in defecation per person per day.

FCAD = no. FC's passed in defecation per animal per day.

FCHA = no. FC's transferred to water during ablution per person per day.

$ZH_{J_j,t}$ = no. people performing ablution directly into point on day t.

FCHC = no. FC's transferred to water during clothes washing per load of clothing per day.

$UH_{J_j,t}$ = no. of loads of clothing washed in point J_j on day t.

$H_{i,t}$ = no. of persons defecating at place i on day t.

$A_{i,t}$ = no. of animals defecating at place i on day t.

$W_{i,t}$ = vector of parameters describing weather at site i on day t (including temperature, humidity, sunlight).

$\phi^{H}(W_{i,t})$ = proportion of human FC's destroyed per day given $W_{i,t}$.

$\phi^{A}(W_{i,t})$ = proportion of animal FC's destroyed per day given $W_{i,t}$.

$\beta^{H}_{J_j}(W_{i,t})$ = proportion of human FC's washed from site i to water point J_j per day, given $W_{i,t}$.

$\beta^{A}_{J_j}(W_{i,t})$ = proportion of animal FC's washed from site i to water body J_j per day, given $W_{i,t}$.

$F_{J_j,J_o,t}$ = vector of parameters describing movement of water between water point J_j and J_o on day t (including tidal action, flow, rainfall).

$\alpha_{(F_{J_j,J_o,t})}$ = proportion of FC moved from water point J_j to point J_o on day t given $F_{J_j,J_o,t}$.

$\delta_{(F_{J_j,J_o,t})}$ = proportion of FC moved from water point J_o to point J_j on day t given $F_{J_j,J_o,t}$.

If we take J_o as the water point of interest this model can then be expressed as:

$$
\begin{aligned}
{}_{J_o,t} = \Big[\Big[& FC_{J_o,t}\left(1-\gamma(v_{J_o,t-1})\right) + YH_{J_o,t}\cdot FCHD + YA_{J_o,t}\cdot FCHD \\
& + XH_{J_o,t}\cdot FCHB + XA_{J_o,t}\cdot FCAB + UH_{J_o,t}\cdot FCHC + ZH_{J_o,t}\cdot FCHA \\
& + FCHD \sum_{\forall i} \Big[\Big[\Big\{\sum_{\forall m} H_{i,t-m} \prod_{l=1}^{m} \{[1-\sum_{\forall j} \beta^{H}_{J_j}(w_{i,t-l})][1-\phi^{H}(w_{i,t-l})]\}\Big]\beta^{H}_{J_o}(w_{i,t})\Big] \\
& + FCAD \sum_{\forall i} \Big[\Big[\Big\{\sum_{\forall m} A_{i,t-m} \prod_{l=1}^{m} \{[1-\sum_{\forall j} \beta^{A}_{J_j}(w_{i,t-l})][1-\phi^{A}(w_{i,t-l})]\}\Big]\beta^{A}_{J_o}(w_{i,t})\Big] \\
& \cdot (1-\gamma(v_{J_o,t})) \cdot \Big(1-\sum_{j=1}^{m}\delta(F_{J_j,J_o,t})\Big) \\
& + \sum_{j=1}^{m} \Big[\Big[\Big\{FC_{J_j,t-1}(1-\gamma(v_{J_j,t-1})) + YH_{J_j,t}\cdot FCHD + YA_{J_j,t}\cdot FCAD \\
& + XH_{J_j,t}\cdot FCHB + XA_{J_j,t}\cdot FCAB + UH_{J_j,t}\cdot FCHC = ZH_{J_j,t}\cdot FCHA \\
& + FCHD \sum_{\forall j} \Big[\Big[\Big\{\sum_{\forall m} H_{i,t-m} \prod_{l=1}^{m} \{[1-\sum_{\forall j} \beta^{H}_{J_j}(w_{i,t-l})][1-\phi^{H}(w_{i,t-l})]\}\Big]\beta^{H}_{J_j}(w_{i,t})\Big] \\
& + FCAD \sum_{\forall i} \Big[\Big[\Big\{\sum_{\forall m} A_{i,t-m} \prod_{l=1}^{m} \{[1-\sum_{\forall j} \beta^{A}_{J_j}(w_{i,t-l})][1-\phi^{A}(w_{i,t-l})]\}\Big]\beta^{A}_{J_j}(w_{i,t})\Big] \\
& \cdot (1-\gamma(v_{J_j,t})) \cdot \propto (F_{J_j,J_o,t})\Big]\Big]
\end{aligned}
$$

The forms of the functions can easily be changed to reflect known or hypothexized non-linearties or interactive effects. These equations will be built into a simulation model. Parameter values will be assigned on the basis of the appropriate independent estimates. A FORTRAN program will be written on the basis of this model structure to simulate the consequences to FC and FS presence of the assumed form and parameter values. The model will be calibrated using data from the longitudinal surveillence of natural water bodies.

By formal analysis using this model, we should be able to assess the variability of FC and FS concentrations under different conditions and, thus, on their utility and that of the FC:FS ratio as indices of fecal water pollution. Sensitivity analysis will also be performed to evaluate the reliability of these indices given uncertainties in out knowledge of certain behavioral, physical or biological factors. The model will also be used to a limited extent to predict the effect of changes in of the parameters on the level of fecal pollution in a water source.

SIGNIFICANCE

Data on the fecal microbiology of water sources in Bangladesh is of importance in the following contexts:

1. Studies on the efficacy of particular sanitation measures will be much more powerful if a dependable index of microbiological water quality is incorporated in the methodology;

2. An understanding of the mechanisms which control the flow of fecal pollution into natural water sources and of the factors which cause

fluctuations in the concentration of fecal contaminants is a necessa prerequisite in the development of rational proposals for limiting pollution; and

3. Cost-benefit analysis of proposed interventions to interrupt disease transmission would benefit from the availability of a means of estimating the level of pathogenic organisms to which persons using different types of water sources under different sets of conditions are exposed. Measurements of FC and FS concentration and the FC:FS ratio are the best available tools for providing the information needed. However, their validity as indicators and the limiting conditions to their use have not been assessed in Bangladesh. This study aims to establis a firm basis for their use in this area and to provide a preliminary analytical framework for the setting of priorities for further resea on intervention modalities.

F. FACILITIES REQUIRED

1. Office space - no additional spece needed

2. Laboratory space - approximately 8 feet of bench space in Room 111 for duaation of study

3. Hospital resources - none

4. Animal resources - none

5. Logistical support -

   a. Transport Dacca-Matlab-Dacca -- 1 time/month

   b. Country boat -- 7 days/month

   c. Car transport in Dacca -- 60 miles/month

6. Major items of Equipment - none

7. Other - none

F. COLLABORATIVE ARRANGEMENTS

None

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SECTION III - BUDGET

A. DETAILED BUDGET

1. PERSONNEL SERVICES

Name	Position	Percent of effort or number of days	Annual Salary	Project Requirements TAKA	Project Requirements DOLLARS
William Spira	Investigator	10%	$ 39,732		3,972
John Briscoe	Investigator	10%	$ 27,500		2,750
Anwaral Huq	Res. Asst.	180d	Tk 16,756	11,729	
---	Lab. Attendent	50d	Tk 7,200	1,380	
---	Male FA	20d	Tk 18,464	1,420	
---	Female FA(5)	20d	Tk 18,464	7,100	
			Sub Total:	21,629	6,722

2. SUPPLIES AND MATERIALS

Items	
Filtration supplies	150
Membrane filters	2,000
Media and Chemicals	100
Plostiet glassware	100
Office supplies and stationery	50
Sub Total:	2,400

			Project Requirements TAKA	DOLLARS
3.	EQUIPMENT			
	None		———	———
		Sub Total:		
4.	PATIENT HOSPITALIZATION			
	None		———	———
		Sub Total:		
5.	OUTPATIENT CARE			
	None		———	———
		Sub Total:		
6.	CRL TRANSPORT			
	Milage Dacca	500 at Tk 1.40	700	
	Milage Baushiaghat	750 at Tk 1.40	910	
	Speedboat	20hr at Tk 98.84	1,976	
	Ferry fees to Baushiaghat	20 x Tk 20	400	
	Country boat	50d at Tk 6.68	334	
		Sub Total:	4,720	
7.	TRAVEL AND TRANSPORTATION OF PERSONS			
	30% Differential for Anwarul Huq while in Matlab, 70 days		1,332	
		Sub Total:	1,332	
8.	TRANSPORTATION OF THINGS			
	Import of supplies	2400 x 0.25		60
		Sub Total:		

		Project Requirements	
9.	RENT, COMMUNICATIONS & UTILITIES	TAKA	DOLLARS
	None		
	Sub Total:		
	PRINTING AND REPRODUCTION		
	Printing forms	300	
	Xerox costs	300	
	Publication costs		250
	Sub Total:	600	250
11.	OTHER CONTRACTUAL SERVICES		
	Payments to volunteers and village boys	300	
	Sub Total:	300	
12.	CONSTRUCTION, RENOVATION, ALTERATIONS		
	None		
	Sub Total:		

B. BUDGET SUMMARY

Category	Project Requirements TAKA	DOLLARS
1. Personnel Services	21,629	6,722
2. Supplies and Materials		2,400
3. Equipment		
4. Patient Hospitalization		
5. Outpatient Care		
6. CRL Transport	4,720	
7. Travel and Transportation of Persons	1,332	
8. Transportation of Things		
9. Rent, Communications & Utilities		
10. Printing and Reproduction	600	
11. Other Contractual Services	300	
12. Construction, Renovation, Alterations		
Total:	28,581	9,978
Total $:	11,877	

Conversion Rate: $ 1.00 = Tk 15/