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EPIDEMIOLOGY OF PERSISTENT AND CHRONIC DIARRHEA IN CHILDREN OF RURAL GUATEMALA

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I. INTRODUCTION

A. Scope of Consultation

The purpose of this consultation was to provide technical assistance to Dr. Jose Ramiro Cruz of INCAP in the design of field procedures and data collection instruments required for implementation of the study "Epidemiology of Persistent and Chronic Diarrhea in Children of Rural Guatemala". This study is a research component of the INCAP project "Oral Rehydration Therapy, Growth Monitoring, and Nutrition Education". The study has been the subject of a previous PRITECH consultation by Dr. Robert Black, who provided on-site epidemiologic review of the initial study protocol.

B. Purpose of the Study

This will be a three-year longitudinal prospective study of persistent and chronic diarrhea among infants and young children in a rural area of Guatemala. As now written, its objectives are to determine the following:

1. The age-specific incidence of persistent and chronic diarrhea.
2. The impact of persistent and chronic diarrhea on subsequent health and nutritional status.
3. The effect of specific potential risk factors on occurrence of persistent and chronic diarrhea; risk factors to be evaluated are:
   a) etiologic agent;
   b) type of initiating diarrheal illness (watery, dysenteric, presence of fecal leukocytes, etc.);
   c) nutritional status at onset of diarrhea;
   d) number and duration of antecedent episodes of diarrhea and other infectious illnesses.

e) feeding practices before and during diarrhea (breast-feeding; feeding of other milks; carbohydrate, protein, and lectin content of diet; carbohydrate malabsorption-stool reducing substances; level of fecal contamination of foods given);

f) treatments given during directly preceding diarrheal illness;

g) cell-mediated immunity status.

II. STATUS OF STUDY

In general, at the time of this visit the study remains in a protocol stage. One important step toward implementation which has been accomplished is agreement by the Chief of the involved Health Area (Sacatepequez) to conduct the study in the chosen field area. Additional approval steps to be accomplished include transmission of official clearance by the National Ministry of Health to the Chief of the Health Area, final clearance at the Health Area level, and acceptance of the study by officials of the Municipio chosen as the study area.

The protocol is in essentially a final form, having been reviewed at several levels within INCAP as well as by PRITECH. In response to the recommendations of Dr. Black and the internal reviewers, the following modifications have been made.

1. Pathogen transmission studies have been dropped.
2. The study has been expanded to include children aged 0-36 months.
3. In addition to the cohort of 150 newborns to be enrolled sequentially during the first 15 months of study, a panel of 150 children aged 0-30 months, living in different households, will be recruited at the beginning of the study. These panel children will be followed until

they reach age 36 months, providing a more even distribution of study effort over three years. Assuming even age distribution of this panel, its inclusion provides an additional 262 child-years of observation, yielding a total of 619 child-years. This sample has been estimated to be adequate to evaluate risk factors of interest, with power to detect significant associations at an estimated proportion of diarrhea cases lasting > 14 days of 0.2, and relative risk of 2.

4. Anthropometric data will be collected routinely each month, as well as at the onset of diarrhea, each week for diarrhea episodes lasting > 7 days, and at the end of the episode (> 3 days without diarrhea).

5. The protocol acknowledges an intention to study feeding patterns and changes in these patterns during diarrhea, with special attention to weaning foods and breast-feeding. However, no detail is provided on how specific foods of potential interest will be identified or quantitated. In addition, it is planned to collect these data only on the 150 sequentially enrolled cohort children. Although not described in the protocol, a preliminary survey of feeding practices will be conducted at the time of enrollment of the 150 panel children; to determine bacterial contamination levels, a weekly sampling of food items from 50 of the 150 panel households will be conducted during the first two months of study.

6. Use of antibiotics (and other treatments) during and immediately prior to diarrhea will be included as a risk factor.

7. Immunization with BCG will be administered to maximize effectiveness of the cell-mediated immunity testing.

8. Scheduled collection of stools from controls (well study children) will occur every 2 months.

9. With the exception of the use of ELISA for E. coli ST and C. difficile toxin, suggested laboratory methodology changes have been made.

10. Documentation of relevance of the risk factors chosen for study has been improved.

11. A study timetable and general plan of analysis have been added.

12. Informed consent forms and provision for appropriate treatment for severe or dysenteric infection with identified pathogens, and hospital referral for severely ill children and those with diarrhea lasting > 21 days. While these treatments may modify the occurrence of prolonged diarrhea, they were required by INCAP's Committee for the Protection of Human Subject and are ethically demanded.

Methodology

Three approaches were utilized to assess the support and resources within INCAP available for this study and to assist in moving it toward realization:

- meetings with responsible and participating INCAP staff;
- detailed review and discussion of plans and procedures for implementation of the study;
- design of instruments for data collection.

III. OBSERVATIONS AND CONCLUSIONS

A. Meetings With INCAP Staff

One or more meetings were held with each of the following persons.

1. Dr. Luis Octavio Angel, Director, INCAP.
2. Dr. Jose Villar, Chief, Division of Nutrition and Health.
3. Dr. Maarten Immink, Chief, Division of Food and Nutrition Planning.

4. Dr. Hernan Delgado, Chief, Section of Interventions in Health and Nutrition, Division of Food and Nutrition Planning; Project Director, Oral Rehydration, Growth Monitoring, and Nutrition Education Project.
5. Dr. Jose R. Cruz, Chief, Section of Nutrition, Infection, and Immunology, Division of Nutrition and Health; Principal Investigator.
6. Dra. America de Fernandez, Section of EPidemiology, Division of Nutrition and Health.
7. Lic. Gilda Pareja, Biostatistician, Division of Nutrition and Health
8. Lic. Elena Hurtado, Anthropologist, Division of Food and Nutrition Planning.
9. Srta. Bertha Garcia, Field Project Supervisor, Division of Food and Nutrition Planning.

I formed several impressions as a result of those meetings. First, INCAP appears to be a relatively dynamic organization with a strong sense of its mission and an atmosphere of mutual coordination. Within this context, there is a strong commitment to support Dr. Cruz's study. Most importantly, most of the knowledge, skills, and experience required as resources for such a study exist within INCAP. If these resources are effectively utilized and coordinated, the study will be likely to succeed.

B. Review and Discussion of Plans and Procedures for Implementation

These discussions primarily involved myself and Dr. Cruz. Also involved in discussions of specific areas were Dr. Delgado (field project management resources), Lic. Pareja (data management, computer capabilities, data analysis), Dr. Luis Gonzaga Elias (nutritional

content of indigenous food items), and Lic. Hurtado (sociocultural background, child care and feeding practices, and indigenous medical practices).

C. Specific Areas of Review

1. Study site and logistics. The chosen study site is Santa Maria de Jesus, a municipio of approximately 8000 persons located in the highlands above Antigua, roughly 50 minutes by car from INCAP in dry weather. Evaluation included a visit to the site with Dr. Cruz and review of socioeconomic studies of the municipio completed by the University of San Carlos in 1982. The municipio contains predominantly households supported by small-scale subsistence agriculture, supplemented by cash crop farming and agricultural labor on neighboring fincas; the population is almost entirely Indian. In these characteristics, this study site is representative of a substantial portion of the population of Guatemala; aside from the culture-specific attributes of its Indian population, it is also representative of the socioeconomic conditions of a substantial part of the population of Central America and Mexico.

Certain specific characteristics of the study site are of importance. The municipio contains a relatively stable population. Younger age groups predominate in the population structure, with approximately 30% of the population under 9 years of age. Education and literacy levels are low, and children may fail to attend school because their labor is needed in the fields. Breast-feeding is the rule, with introduction of weaning foods within the first 6 months of life and complete weaning usually by 2 years of age. Diet consists mostly of maize and beans, supplemented by some fruits and vegetables, less frequently by meat, eggs, and dairy products. Previous INCAP determinations

have documented the diet of a comparable population in the Sacatepequez Health Area to be below child requirements of calories, proteins, and vitamins A and C. Water is distributed to several public outlets in the municipio; at most of these water is available only at restricted hours, with greatest scarcity late in the dry season. As the result of a government sponsored latrine-building program, most households have latrines; however, non-utilization of these is common. In some areas waste water from households runs into gullies in the streets.

The municipio has a health clinic staffed by a nurse, a senior medical student (not in attendance at the time of our visit), and a health technician. Over the past several years there have reportedly been less than optimal services offered at the health clinic, with resultant underutilization of the clinic. Reportedly this situation has somewhat improved, but the clinic was not busy during our visit. This situation will need to be taken into account when considering how the study will interact with the community, especially in regard to referral of sick children. Substantial care will be required to establish confidence in the community.

Some health services are also offered by nuns and by a "Save the Children" program. There is also a network of neighborhood health promotors. Oral rehydration therapy has been established; however, intermittent shortages of ORS packets have occurred. Clinic records show treatment of approximately 250 cases of diarrhea in the clinic in 1984. These records also list the principal causes of childhood mortality as "respiratory infections" (25%), "malnutrition" (23%), and "diarrhea" (22%), these diagnoses were partly ascertained by the clinic and partly by parent report, and their accuracy is therefore questionable.

Nonetheless, they indicate that the goals of this study should be of interest to the population.

With regard to logistics, the distance of this site from INCAP will permit daily transportation of field workers to and from Guatemala City; this will minimize logistic, communication, supervisory, and field worker recruitment difficulties. The dirt road from Antigua to the municipio is reportedly adequately maintained to permit travel without substantial difficulty during the rainy season. There is a high likelihood of being able to establish a field project post in a small building at the site for field workers, equipment, forms, etc.

An important finding during our visit is that recently the municipio has experienced a problem with several ladino persons who came to the village presenting themselves as "natural medicine" doctors and promising medicine and treatment. These persons were subsequently found to be stealing corn and beans from the people. Consideration of this experience will be important, since the first phases of the INCAP project will include inquiries about feeding and collection of food samples; remuneration for food samples may be indicated.

2. Field worker requirement. Present plans call for 6 field workers. The peak number of study children (occurring in the sixteenth month) will be 255. This will require 85 visits each day of a six day work week; of these, an estimated 10-15 visits will involve initial or follow-up evaluation of children with diarrhea. These visits will require 4-5 hours daily per field worker. Allowing for transportation, specimen processing, and record keeping, the number of workers appears appropriate, but may require supplementation if anthropometry, seasonal peaks of diarrhea incidence, transportation difficulties, or administrative duties substantially increase their time requirement.

A pool of potential field workers, many with appropriate field experience, exists within INCAP. Field workers will require training and demonstrated skill in interviewing, questionnaire completion, anthropometry, evaluation of diarrhea and dehydration, oral rehydration therapy, specimen collection, and record keeping. Dr. Cruz is aware of these training requirements and has plans to provide appropriate training sessions, since resources for this training (such as Srt. Garcia) are available within INCAP.

3. Field project management. Dr. Cruz and I discussed in detail the importance of providing middle management for the study in the form of a field supervisor, and the functions of such a person. This supervisor will require an understanding of study goals and methods, experience in field work (or related experience), ability in leadership and management, sensitivity to potential problems, and ability to solve such problems. The field supervisor will be responsible for overseeing and guiding the field data collection, supervising the field workers, and checking and verifying data collected. To carry out these functions, the supervisor will require an appropriate level of authority and an open channel of communication with the principal investigator. Dr. Cruz strongly feels this management position to be essential to conducting the study, and I concur. Models for such supervisors exist within INCAP, and he will begin the search for an appropriate person in the near future.

4. Field procedures. Dr. Cruz and I reviewed plans for study procedures including enumeration of the study population, randomization and sample selection, visit schedule, food sample collection, anthropometry, processing of forms, and record keeping. In some of these areas the experience of Lic. Hurtado and Srta. Garcia were of particular relevance. At this point,

realistic and appropriate plans exist for each of these procedures. A uniform approach to eliciting responses to questionnaire items will be developed for use by all field workers.

5. Timetable. The relative time distributions set forth in the revised protocol are generally still appropriate. Minor changes include recruitment of the panel children before execution of the food contamination evaluation phase (since this phase will be carried out in a subset panel of households) and extension of the food contamination phase over two months. An obvious major change is the projected starting date: given the clearances and personnel issues to be resolved, it appears that initiation of the study cannot realistically be expected until May or June. Given the importance of preparatory arrangements for a study of this magnitude and duration, I feel this shift is appropriate. Dr. Cruz and I have also discussed repeating the food contamination study in the dry season, since foods used and levels of environmental contamination may vary seasonally.

6. Pilot testing. Pilot testing of the questionnaires and the field procedures for the longitudinal study will be conducted during the two month initial food contamination evaluation phase.

7. Validity and bias. Dr. Cruz demonstrates a strong awareness of the need to validate data collected in the course of such a study. The principal approach to validation will be observation, an approach which is easier in such a longitudinal community-based study than in clinic-based or case-control studies. In addition to cumulative observation of actual practices, specific data will be validated, for example asking to see food items reportedly fed and comparing reported status of the child with observed status. The field supervisor will also periodically verify data collected.

Substantial potential for biases exist in both the study population and the field workers. Dr. Cruz is aware of these and has incorporated them into his data collection strategy. These issues will be discussed with the field workers and their supervisors during orientation and periodically thereafter.

8. Specimen collection and testing. Dr. Cruz has substantial experience regarding methodology for collection, processing, and transport of specimens in field studies. Appropriate provisions have been made for specimen collection at the household level, transport media, temperature stabilization, labeling, and record keeping. Quality control procedures have been developed, using stool specimens "seeded" with known pathogens handled and transported under field conditions.

As noted, laboratory testing procedures are generally appropriate. One modification to be considered would be replacement of much of the labor intensive E. coli toxin, adherence, invasiveness, and serotyping work with the use of DNA probes. Highly sensitive and specific commercial oligonucleotide probes now exist for LT and the 2 types of human ST. Additional probes exist for EPEC, invasiveness, and Shiga-like (Vero) toxins I and II. Several such probes have already been applied in large field studies in Thailand by Echevarria. This technology is useful in testing large numbers of specimens; its utilization requires the capability to handle radioactive isotopes (^{32}P or ^{35}S). Dr. Cruz is in contact with Dr. Barbara Murray of the University of Texas, who has experience in laboratory and field application and development of these probes.

9. Data management. In the past the Division has encountered problems in this area. To address these problems, they have recently hired a full time data entry clerk and a full time, well trained biostatistician

(Lic. Pareja). These staff should provide adequate infrastructure for management of data entry and editing, and to conduct periodic interim analyses to assure data quality and to evaluate intended analytical approaches using the data set.

Computer hardware capabilities are adequate at this time. The Division has two terminals accessing a Hewlett-Packard 3000 minicomputer with a large storage capacity and 1 megabyte RAM. The Division also has an IBM PC/AT with 20 megabyte storage and 1.3 megabyte RAM, and an IBM PC with 256K RAM. A software program has been ordered which will allow interaction of the PC/AT and the HP3000. In addition, the Division plans to buy a Bernoulli box for multiple hard disk data storage; this will provide a greatly expanded data file storage capacity.

Software is likewise adequate, if not optimal. SPSS and BMDP are available in the HP3000. SPSS (PC) and a modified BMDP are available for the PC/AT. Several up-to-date statistics, data management, and data entry programs are available. One useful addition might be SAS, which would add multivariate analysis capabilities not included in the available software; I believe a PC/AT compatible version of SAS is or will soon become available. One possible limitation will be access to PC terminals; at present terminals are shared between data entry and word processing. This may become a significant bottle-neck in the future.

10. Analysis. Several meetings were held with Dr. Cruz and Lic. Pareja to discuss analysis considerations, primarily in the context of data organization and questionnaire design. These discussions focused on issues of partitioning the substantial amount of data into manageable and analyzable sets, transforming individual items into groups meaningful for analysis (e.g. food items

consumed into meaningful dietary categories), alternative approaches to determining relative risk associated with specific potential risk factors, and the issues of interdependence of variables (such as serial measurements of the same child, and risk factors for episodes of prolonged diarrhea in children who also experience shorter episodes of diarrhea). Satisfactory approaches to these issues were developed to allow drafting of questionnaires; further consideration of these analysis issues will be required on the part of Dr. Cruz and Lic. Pareja before final questionnaire formats and data entry programs are written.

D. Design of Instruments for Data Collection

Based on the considerations discussed above, several data collection forms were determined to be necessary. The format and content of each of these were discussed in detail, and drafts of the most important forms were constructed. Additional specific information regarding local feeding practices and treatments for child illnesses will be required before versions suitable for pilot testing can be drafted. The following are the forms to be used.

1. Household census form (population enumeration).
2. Study household/child socioeconomic characteristics (enrollment).
3. Study household/child, feeding/illness treatment practices (enrollment).
4. Food sample specimen control form.
5. Daily child symptom and treatment record.
6. Monthly summary of child illness, treatment, feeding, anthropometry, and other periodic data.
7. Diarrhea episode form.
8. Stool specimen control form.
9. Stool specimen laboratory processing and result forms.

These forms were designed to permit ready access to all data regarding a particular child and to form a continuous longitudinal record of significant events during the course of each child's participation in the study, while permitting data to be analyzed in terms of risk factors, diarrhea episodes, age, and other attributes.

IV. GENERAL CONCLUSIONS AND SUMMARY

INCAP is an institution uniquely qualified to undertake a study such as this. It offers a body of experience in field study and laboratory research in diarrheal disease and nutrition. Within the Division of INCAP responsible for this study exists most of the resources and facilities needed to operationalize and complete this study.

One limitation on execution of this study is provision of project management. This limitation will be partly corrected by appointment of a competent field supervisor. However, substantial constraints exist on the time available to Dr. Cruz to participate in the field management of such a study. While Dr. Cruz has important experience in field studies, substantial understanding of epidemiologic principles, and excellent abilities as a problem solver and communicator, his primary training and responsibilities are not in epidemiology, but in laboratory science. As his laboratory skills and facilities become increasingly occupied with this and other projects, Dr. Cruz will have even less time available for management of field research. Epidemiologic and field management assistance will be particularly important during the initiation of field operations.

Another important factor in success of this field study will be the establishment of trust and cooperation with the municipio and the population to be studied. Our experience and feedback from INCAP anthropologists

suggest that the population (like that of many such settings) is distrustful of outsiders and has had some negative experience with real or purported health personnel. I believe that in addition to time required to recruit personnel and obtain clearances, time should be invested in exploring optimal ways to frame the study within the community, in gaining community support, and in searching for mechanisms to provide benefit to the community in a way which the community will perceive as beneficial. Failure to invest this time initially may result in poor data quality or even failure of the study.

Finally, additional anthropology and nutrition expertise will be required to ensure correct ascertainment of feeding practices and child illness treatments, and to utilize specific food information obtained in a meaningful dietary assessment. This expertise is available within INCAP.

A. Principal Recommendations

1. The study timetable should be adjusted to initiate field activities in June, 1986; if additional time before start up is required to establish secure community relations, this time should be taken.

2. Provisions should be made to provide this study with intermittent or continuous project management and epidemiologic support; support will be particularly useful in July, 1986, when routine field procedures are expected to be initiated.

3. INCAP expertise in anthropology and nutrition should be recruited to finalize field procedures and data collection instruments.

B. Secondary Recommendations

1. Consideration should be given to transferring DNA probe technology to Dr. Cruz's laboratory for *E. coli* pathogenicity determinations.

2. Future review of computer terminal access is indicated; the Division may need to purchase an additional PC to sustain the process of continuous data entry required by this and other studies.