Report of the Twelfth Meeting of the

STEERING COMMITTEE OF THE
SCIENTIFIC WORKING GROUP ON BACTERIAL ENTERIC INFECTIONS:
MICROBIOLOGY, EPIDEMIOLOGY, IMMUNOLOGY, AND VACCINE DEVELOPMENT

(Geneva, 10-13 September 1985)

CONTENTS

<table>
<thead>
<tr>
<th></th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Consideration of the report of the eleventh meeting</td>
<td>2</td>
</tr>
<tr>
<td>2. Review of follow-up action and ongoing activities</td>
<td>2</td>
</tr>
<tr>
<td>3. Budget</td>
<td>5</td>
</tr>
<tr>
<td>4. Review of renewal applications and final reports</td>
<td>6</td>
</tr>
<tr>
<td>5. Review of new and revised proposals</td>
<td>6</td>
</tr>
<tr>
<td>6. Overview of projects funded to date</td>
<td>8</td>
</tr>
<tr>
<td>7. Site visits and training</td>
<td>8</td>
</tr>
<tr>
<td>8. Other matters</td>
<td>8</td>
</tr>
<tr>
<td>9. List of participants</td>
<td>8</td>
</tr>
</tbody>
</table>

The twelfth meeting of the Steering Committee (SC) of the Scientific Working Group (SWG) on Bacterial Enteric Infections was held in Geneva from 10 to 13 September 1985. The participants are listed at the end of the report.

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INTRODUCTION

The Committee reviewed the revised Guidelines for Management of Research Activities and noted that, from 1 January 1986, the SWGs and SCs on Bacterial Enteric Infections and Viral Diarrhoeas would be combined in a single SWG: Immunology, Microbiology and Vaccine Development (IMV). Also, a new SWG would be created on Epidemiology and Disease Prevention (EDP). Except for epidemiological studies, the responsibilities of the SWG/IMV would resemble those of the Steering Committees from which it derived.

1. CONSIDERATION OF THE REPORT OF THE ELEVENTH MEETING

The Committee reviewed and approved the minutes and report of its eleventh meeting and of its extraordinary meeting held on 7 June 1985 in Stockholm.

2. REVIEW OF FOLLOW-UP ACTION AND ONGOING ACTIVITIES

2.1 Follow-up action on proposals considered at the last meeting

The Committee considered the follow-up action taken on proposals considered at the last meeting.

It approved the awarding of continued support for the following projects:

- 84036 - Field trial of oral B-subunit/wholecell and wholecell cholera vaccines - J. Clemens, International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka ($50,000)

- 82017 - Aetiology of acute diarrhoeal diseases. Pilot study in a sub-saharan area, Sokoto State, Nigeria - J. Osisanya, University of Sokoto, Sokoto, Nigeria ($15,150)

It also approved the awarding of support to the following new projects which had been reviewed by the SC at its last meeting:

- 85087 - Safety, infectivity, immunogenicity and transmissibility of attenuated Aro<sup>−</sup>, Pur<sup>−</sup>, S. typhi strains 541Ty and 543Ty - M. Levine, Center for Vaccine Development, Baltimore, Maryland, USA ($26,900)

This is a study of the safety and immunogenicity of a new candidate S. typhi vaccine in volunteers. The vaccine is an Aro<sup>−</sup>, Pur<sup>−</sup>, genetically defined mutant produced by Dr B. Stocker.

- 84086 - Food and water contamination as possible risk factors of acute diarrhoea during the rainy season in Rangoon, Burma - Aung Myo Han, Department of Medical Research, Rangoon, Burma ($35,000)

This is an epidemiological study to determine the role of contaminated food in the transmission of bacterial enteric pathogens during the summer in Rangoon, Burma.

2.2 Escherichia coli toxin testing (LT and ST)

2.2.1 Anti-LT sera

The second lot of purified anti-LT obtained by WHO was reported to have satisfactory stability; for maximum shelf life, the lyophilized antiserum should be stored refrigerated. The lot of anti-LT serum produced by the Swiss Serum and Vaccine Institute (SSVI) was reported to be satisfactory for use in the GM<sub>1</sub> ELISA and the Biken test. The serum correctly identified all LT-producing E. coli from a panel of 50 strains and gave no false positive reactions. It could be recommended for use by investigators being supported by the Programme.
2.2.2 Virion test kit for LT (Biken test)

It was noted that the problem of dark or cloudy agar with the Virion kit could not easily be corrected because the agar contained glucose which darkened during autoclaving; the SC agreed that this limited the value of the kit. While other agars which did not darken when prepared could be used in the Biken assay (for example, tryptic soy agar), it was uncertain whether they would be satisfactory for the detection of hypotoxicogenic strains. It was agreed that the WHO Collaborating Centre for Phage-Typing and Resistance of Enterobacteriae would evaluate several commercial agars using hypotoxicogenic LT-producing ETEC.

2.2.3 ST ELISA assays

Evaluation of the direct ELISA for ST of Dr F. Klipstein had proved unsatisfactory for the detection of ST but (paradoxically) had detected about 70% of LT-producing E. coli. Moreover, the antiserum provided had efficiently neutralized LT. The SC concluded that there was no immediate prospect of developing a satisfactory direct ELISA for ST, several other investigators having also failed in their attempts to develop such an assay. In contrast, the competitive ST-ELISA being developed by Dr R. Giannella (Project No. 82068) had shown 100% agreement with a radio-immunoassay and the suckling mouse assay for ST when evaluated using a panel of 100 E. coli strains. The possibility of developing this test commercially was considered attractive, and it was suggested that the test might be improved by using synthetic ST in place of purified native ST.

2.3 Campylobacter serotyping

The Committee reviewed the status of the ongoing evaluation of the campylobacter serotyping scheme developed by Dr R. Lior, Laboratory Centre for Disease Control, Ottawa, Canada. It estimated that Dr Lior would receive about 750 strains from the 8 centres participating in the evaluation and agreed to provide him with up to $15 000 in additional funds for the typing of those strains.

The SC noted that antisera for typing C. jejuni by the Lior scheme would be produced by a commercial company in Belgium (Interlaboratoire, Brussels) in the near future. In addition, Dr Lior had agreed to cooperate with the WHO Collaborating Centre for Campylobacter jejuni in developing materials to assist interested laboratories in making their own antisera. The materials would include guidelines for the production of C. jejuni antisera, type strains for the 20 most frequently isolated serotypes, and antisera to confirm each of the type strains, and would be distributed by the Collaborating Centre. The SC recommended that development of the materials proceed as rapidly as possible.

2.4 Phage typing of V. cholerae O1

An evaluation of the phage-typing scheme developed in Rostov, USSR, conducted by the WHO Collaborating Centre for Phage-Typing and Resistance of Enterobacteriae, had shown that about 90% of V. cholerae O1 from various geographical areas were typable and that prevalent types differed markedly according to whether the strains were obtained in Africa or Asia. The Committee recommended that the scheme developed at the National Institute of Cholera and Enteric Diseases be evaluated by the Collaborating Centre in the same manner as the Rostov scheme. If that evaluation proved satisfactory, both schemes should then be tested in two other laboratories using a single set of strains collected from different geographical sites.
2.5 Multicentre study

2.5.1 Institutes

Burma (Department of Medical Research, Rangoon)

The preliminary results of a collaborative study conducted by the Department of Medical Research, Rangoon, and the Osaka Prefectural Institute of Public Health, Japan, on the bacterial etiology of acute diarrhoea were reported. Stools from 28 infants and children under 5 years of age with acute diarrhoea and 19 healthy controls had been studied. Pathogens had been isolated from 50% of the diarrhoea stools and 32% of control samples. Interestingly, the samples from cases yielded 2 isolates of EIEC and one of Plesiomonas shigelloides. Otherwise the results were similar to those obtained in the multicentre study. The Committee looked forward to reviewing the final results of the study.

Pakistan (National Institute of Health, Islamabad)

A microbiologist from the National Institute of Health had completed 6 weeks of training at the Central Public Health Laboratory and the Central Middlesex Hospital, London, UK, and had returned to Pakistan; his training had focused on isolation and typing of salmonella and campylobacter. The SC recognized the problems that had limited progress in the study and recommended that it be started again from the beginning.

Mexico (Hospital Infantil, Mexico City)

The SC approved the request for supplementary funds ($4000) to support the analysis of the results of the study. It asked that the computer programme developed for that purpose be provided to the Secretariat for use by other multicentre projects.

China (Hygiene and Anti-Epidemic Center, Shanghai)

The SC approved the final report on the project. Noting the high incidence of shigella isolates in the study, it suggested that studies on the transmission of shigella in Shanghai would be of interest.

2.5.2 E. coli serotyping

The Committee reviewed the results of the initial evaluation by the WHO Collaborating Centres for Phage-Typing and Resistance of Enterobacteriaceae and Reference and Research on Escherichia and Klebsiella of the new EIEC and EPEC polyvalent pools produced by DIFCO. It noted that, with the exception of cross-reactivity with serogroup 028ac by the poly C pool, the specificity and sensitivity of the EIEC polyvalent antiserum pools were satisfactory. As regards the EPEC polyvalent pools, one of the Centres reported that the poly C pool was completely inadequate, failing to identify most serotypes included in that pool. The Committee expressed disappointment that DIFCO had decided not to produce monovalent sera as the polyvalent sera provided had little practical value, their specificity when tested against all serogroups of E. coli being unknown. It agreed that efforts should be made to identify another company that might prepare monovalent sera.

2.6 Cholera vaccines

2.6.1 Centre for the Trial of Vaccines against Infectious Diseases, Bangkok, Thailand

The Committee considered a proposal for the first study to be done at the Centre:
85122 - Immunogenicity of two formulations of killed whole-cell Vibrio cholerae and B subunit vaccine in Thai volunteers - Srichareon Migasena, Vaccine Trial Centre, Mahidol University, Bangkok, Thailand

The study will compare systemic and local immune responses in volunteers immunized orally with a combined whole-cell V. cholerae and B-subunit vaccine. The field trial lot of vaccine will be used in two schedules, one resembling that used in early volunteer studies at the University of Maryland, the other identical to that in the current field trial at ICDRB. The SC approved a minimum budget of $25,000 for the project.

As regards future studies at the Centre, the SC suggested a comparison of the immunogenicity of orally administered procholeragenoid, B subunit, and B subunit combined with small amounts of cholera toxin. The purpose would be to determine (1) whether cholera toxin, given in doses below the diarrhoea threshold, could act as an adjuvant, enhancing the immune response to B subunit, and (2) whether the immunogenicity of B subunit plus CT was comparable to that of procholeragenoid.

2.6.2 Pasteur Vaccine-BioMérieux

The SC was informed that trial lots of the candidate cholera vaccine of Professor Dodin were now being produced and would soon be tested for safety and immunogenicity in French volunteers. The vaccine was expected to be ready early in 1986 for evaluation in volunteers at the University of Maryland. The SC agreed, in principle, to give partial support to the volunteer study. It also considered that the vaccine formulated in enteric-coated microgranules should be practical for administration to infants and small children.

2.7 Typhoid vaccines

2.7.1 Ty21a vaccine trial, Chile

The Committee approved support for at least one more year for continued surveillance in the 3 field trials being conducted with this vaccine in Santiago (ID Nos. 80004, 83042, 84029). Depending on the results available in April 1986, a booster immunization might be planned for half of the participants in Area Occidente and possibly Area Norte; surveillance in Area Sur would be terminated after 12 more months.

2.7.2 Typhoid vaccine trial in Pajau, Sumatra, Indonesia

The SC was informed that studies to prepare the field trial area would be completed in early 1986 and that the Indonesian Ministry of Health wished to conduct a placebo-controlled trial later in the year. In that regard, SSVI was developing a formulation of the Ty21a vaccine which was reconstituted from lyophilized before administration and thus resembled the vaccine formulation originally tested in Egypt. SSVI expected the vaccine to be available for field trial by mid-1986. The SC reaffirmed that it would support only a placebo-controlled trial, preferably one in which the new formulation was compared either with parenteral vaccine or the enteric-coated vaccine.

2.7.3 Studies to determine optimal sites for typhoid vaccine trials in Indonesia

The progress report on studies to develop a second typhoid vaccine field trial site in Java (ID No. 83003) described an attack rate of 630 per 100,000 per year for the age-group 3 to 19 years, using less-than-optimal culture methods; the studies were continuing although additional funds had not been requested. The SC decided that no specific plans should be made at present for a vaccine trial at the Java site.

3. BUDGET

The Committee reviewed the funds remaining for 1985, as indicated below. It anticipated that the amount available for contracts would be sufficient for the projects likely to be supported during the remainder of the year.
4. REVIEW OF RENEWAL APPLICATIONS AND FINAL REPORTS

The Committee reviewed 1 final report, 14 applications for a renewal of support, and 6 progress reports in which further support was not requested. It agreed to provide additional support for 10 of the 14 applications; for 2 others, more information was requested; and for the remaining 2, further funding was not approved. Three of the approved projects have been described in section 2.7.1. The other 7 approved projects are:

(a) B2156 - Horizontal and longitudinal study on the prevalence of rotavirus and other enteric pathogens in children in Hong Kong - J. Tam, University of Hong Kong

(b) B2029 - A community-based longitudinal study on the impact of an environmental intervention programme on the prevalence of enteric pathogens and the etiology of acute diarrheal disease in a rural area of Nigeria - O. Dosumu-Ogunbi, University of Lagos, Nigeria

(c) B1076 - Epidemiology of cholera: modes of transmission of El Tor cholera in affected areas of Tanzania - F. Mhailu, Muhimbili Medical Centre, Dar es-Salaam, United Republic of Tanzania

(d) B4023 - Antibodies to Vibrio cholerae lipopolysaccharides, adhesins, and toxin in human - Wanpen Chaichumta, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

(e) B2158 - Virulence investigations and diagnostic approach of enteroinvasive Escherichia coli/EIEC - I. Rétyi, Institute of Microbiology, Pécs, Hungary

(f) B1931 - Characterization of E. histolytica surface antigens by monoclonal antibodies - D. Triessl, University of Osnabrück, Federal Republic of Germany

(g) B2085 - Ribosomal Shigella vaccine - V. Levenson, Gabrichevensky Research Institute of Epidemiology and Microbiology, Moscow, USSR

5. REVIEW OF NEW AND REVISED PROPOSALS

The Committee reviewed 14 new or revised proposals, of which:

- 7 were approved for support either as originally submitted or pending minor technical or budgetary modifications;

- 1 was kept in abeyance, pending revision of the proposal;

- 6 were rejected (in one case a new proposal was invited).
One of the approved proposals has been summarized in section 2.6.1. The remaining 6 are briefly described below:

(a) 82166 - Cloning and expression in Escherichia coli of the gene coding for the adhesive antigen of Vibrio cholerae - B. Srivastava, Central Drug Research Institute, Lucknow, India ($14 520)

The study aims to clone the gene for the mannose-sensitive haemagglutinin of V. cholerae into E. coli so that this material may be obtained in pure form and in large amounts.

The Committee considered this project to be of potential importance for efforts to develop a live cholera vaccine.

(b) 83176 - Isolation and characterization of enteroadherent factor of enteropathogenic E. coli (EPEC) and its receptor on human epithelium - G. Schoolnik, Stanford University, USA ($21 000)

It is proposed to identify, isolate, and characterize the adhesive surface protein of EPEC and the mucosal receptor with which it interacts. When this has been done, it is planned to develop an assay for EPEC based upon the interaction of the bacteria with the purified receptor.

(c) 84055 - Etude longitudinale des diarrhées aiguës à Campylobacter et à Shigella chez les enfants vietnamiens dans leur milieu naturel - Duong Quynh Hoa, Centre de Pédiatrie du Vietnam, Ho Chi Minh-ville, Viet Nam ($25 000)

This is a revised proposal for a longitudinal study on infection and reinfection with shigella and campylobacter during the first 3 years of life in Vietnamese infants and children. In addition to determining the incidence and clinical severity of these infections, a major objective is to gain a better understanding of how immunity induced by an initial infection modifies the course of a subsequent infection with the same, or a different, serotype of the organism.

(d) 85072 - Preliminary investigation into genetic differences between invasive and non-invasive symbionts of E. histolytica - J. Ackers, London School of Hygiene and Tropical Medicine, London, UK ($24 000)

The study aims to investigate the genetic determinants of virulence of E. histolytica. Specifically, it proposes to develop methods for the culture of non-invasive E. histolytica, to compare DNA from invasive and non-invasive strains, and to search for specific genes that are related to invasiveness. Ultimately, it is hoped to develop a diagnostic test for invasive E. histolytica based upon identified genetic determinants of invasion.

(e) 85086 - La cryptosporidiose digestive au Rwanda, manifestations cliniques et comparaison des techniques d’identification en milieu tropical - P. de Mol, Université Nationale de Rwanda, Butare, Rwanda ($24 000)

It is planned to carry out epidemiological and clinical studies on cryptosporidiosis in Rwanda. The main objectives are to determine the importance of cryptosporidia as a cause of acute diarrhoea in infants and children, to search for secondary cases of infection in family members, and to investigate possible transmission of the parasite from domestic animals to humans.

(f) 84109 - Development of an LPS-based Shigella dysenteriae 1 vaccine - K. Timmis, Centre Médical Universitaire, Geneva, Switzerland

This is a revised proposal to develop a vaccine for Shigella dysenteriae type 1 infection, focusing on evaluation in animal models of the efficacy of a hybrid vaccine that expresses LPS of S. dysenteriae type 1.
6. OVERVIEW OF PROJECTS FUNDED TO DATE

The Committee reviewed summaries of the projects which had been funded to date by the 3 global SCs. These included summaries of projects supported according to research priority area, the number of projects and total funds awarded according to region and individual countries within each region, and the distribution of projects and funds awarded between developed and developing countries. The SC noted that most of its funded projects concerned epidemiology, laboratory diagnosis, pathogenesis and disease resistance, immune mechanisms, development of new or improved immunogens, and vaccine testing.

7. SITE VISITS

The Committee recommended that site visits be made to institutes in Colombia, Rwanda, and Thailand.

8. OTHER MATTERS

8.1 Cryptosporidiosis

The Committee reviewed recent articles documenting the finding that cryptosporidia cause acute diarrhoea in immunocompetent persons. It considered that further studies to determine the frequency of such infections, their clinical severity, and their modes of transmission should be supported by the Programme in order to determine the importance of this pathogen as a cause of diarrhoea.

8.2 Revised research priorities

The SC was informed that, as part of the reorganization of the research component of the Programme, a modified set of research priorities would be developed. As part of this process, the SC reviewed a draft revision of the BRI research priorities and recommended a number of changes.

9. LIST OF PARTICIPANTS

Members:

Dr J. Holmgren, Department of Medical Microbiology, University of Göteborg, Göteborg, Sweden

Professor L. Le Minor, Director, WHO Collaborating Centre for Reference and Research on Salmonella, Institut Pasteur, Paris, France

Dr B. Rowe, Director, WHO Collaborating Centre for Phage-Typing and Resistance of Enterobacteria, Central Public Health Laboratory, London, UK (Chairman)

Dr D. Sack, International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh

Professor Y. Takeda, Chairman, Department of Bacterial Infections, The Institute of Medical Science, University of Tokyo, Tokyo, Japan

Professor L.R. Trabulsi, Department of Microbiology, Ecola Paulista de Medicina, São Paulo, Brazil

Secretariat:

Dr M.H. Merson, Director, Diarrhoeal Diseases Control Programme
Dr N.F. Pierce, Research Coordinator, Diarrhoeal Diseases Control Programme (Secretary)
Dr I. de Zayas, Consultant, Diarrhoeal Diseases Control Programme