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Editorial Note

THE NEED TO LINK JAPANESE ACADEMIC RESEARCH WITH THE MILLENNIUM DEVELOPMENT GOALS FOR HEALTH

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Tropical Medicine and Health

To achieve health-related Millennium Development Goals (MDGs), Japan has made significant financial contributions. In June 2005, the Japanese government launched the 'Health and Development' Initiative (HDI) (1) to work towards the following specific measures: (a) assistance for strengthening institutional capacity development in the health sector, (b) assistance in areas that reinforce the health sector and cross-cutting actions, and (c) actions towards MDGs that focus on the following three health-related goals: Goal 4 Reducing child mortality; Goal 5 Improving maternal health; Goal 6 Combating HIV/AIDS, malaria and other diseases. Under HDI, the proposed financial contribution will be $5 billion for the period between Fiscal Year (FY) 2005 and 2009. The government has also pledged $500 million to the Global Fund to Fight AIDS, Tuberculosis and Malaria (2), and $158 million to fight against avian and pandemic influenza at the regional and global levels for the coming years (3).

Japan International Cooperation Agency (JICA) has also made technical and other contributions to the health-related MDGs (4). JICA prioritizes human resource development and capacity development with the goal of promoting ownership and sustainability, so that target developing countries can take their own initiatives to develop and improve their health care systems (4). In particular, for the Goals 4 and 5 activities, JICA disbursed approximately $300 to $600 million/year for the period between FY 1996 and FY 2003. For the Goal 6, the amount has steadily increased from approximately $1,2 billion in FY 1996 to almost $2 billion in FY 2003. Technical cooperation projects take a large share of the funding for all three goals, using 39.0% of the total assistance for the goals 4 and 5, and 28.8% for the goal 6 in FY 2003 (4).

What about academic contributions? In the Igaku-chuo-zasshi (Japan Centra Revuo Medicina) database, which was established in 1903 and currently cites over 300,000 articles annually from approximately 2,400 Japanese biomedical journals, only 3 articles were listed with 'millennium development goals' as a key word by the end of August, 2006. Of the three, two were the abstracts from conference presentations. In Medline, as of 6 August 2006, 135 articles were listed with 'millennium development goals' as a key word, however none of the authors or the institutions was in Japan. Although some MDG-related articles with Japanese co-authors may be included in more extensive manual search results, these simple computer searches of both Japanese and international databases suggest that Japanese research in international health is not well connected with the health-related MDGs.

To improve this situation, *Tropical Medicine and Health* expects more contributors to link scientific research with the MDGs. Moreover, to increase further academic contributions, more contributors should seek funding for research to link field activities in international health with the MDGs. If the current situation continues, the considerable financial and technical contributions made by the Japanese towards health-related MDGs will not be recognized in the history of achieving these goals.

(1) Ministry of Foreign Affairs Japan. "Health and Development" Initiative (HDI)-Japan's contribution in achieving the health related MDGs. The Government of Japan, 2005. (available at http://www.mofa.go.jp/policy/health_c/forum0506/hdi.pdf, accessed 13 September, 2006)
(2) Yamamoto T. Japan's contribution in achieving the health MDGs. J Int Health 2005; 20: 63-4.
(3) Yamamoto T. Proactive plan to help contain pandemic influenza in Asia. Trop Med Health 2006; 34: 75-6.
(4) Japan International Cooperation Agency. Our challenge for a better tomorrow: report on JICA's contribution to achieving the Millennium Development Goals (MDGs). Planning and Coordination Department, Japan International Cooperation Agency, 2005. (available at http://www.jica.go.jp/english/about/policy/mdg/pdf/mdgs_erep01.pdf and http://www.jica.go.jp/english/about/policy/mdg/pdf/mdgs_erep02.pdf, accessed 13 September, 2006)

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News Watch

NEW TRENDS IN INTERNATIONAL PUBLIC HEALTH LAUNCH OF A NEW DIPLOMA, MSc & PhD PROGRAMME IN RESEARCH & DEVELOPMENT OF PRODUCTS TO MEET PUBLIC HEALTH NEEDS

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Based on the idea that more human resources are needed in the drug/vaccine/diagnostics research and development field in the developing countries where new products are required to improve their unique health situation different from developed countries, a new educational programme has been launched in Japan. Here, I would like to show briefly its philosophy, strategy and contents. This programme has not been completed, however, we have started a core diploma course that is consisted of seven modules that cover all the steps to develop a new product from a very basic discovery. This course has been made possible by a synergistic cooperation between several good persons from various institutions. Their names and institutions are as follows, Win Gutterrage, Janis Lazdins, Juntra Karbwang (World Health Organisation, Switzerland), JinHong Hu (Second Military Medical University, China), Kesara Na-Bangchang (Thammasat University, Thailand), Chitr Sitthi-amorn (Chulalongkorn University, Thailand), Kiichiro Tsutani (University of Tokyo, Japan), Ivan Valez (University of Antioquia, Colombia), and Kenji Hirayama (Nagasaki University, Japan).

I would like to acknowledge Kazuhiko Mori, Center for Product Evaluation, Pharmaceutical and Medical Devices, PMDA, Japan, Kihito Takahashi, Japanese Association of Pharmaceutical Medicine (JAPHMED), Masaru Iwasaki, GlaxoSmithKline, Tokyo, Japan, Masakatsu Shibasaki, (The University of Tokyo, and The Pharmaceutical Society of Japan, PSJ), Hiroshi Saitoh (President, Nagasaki University) for their great contribution to the diploma course.

Website: http://www.tm.nagasaki-u.ac.jp/hiraken /information/deploma/diploma_top_frame.html

BACKGROUND:

The development of new drugs, vaccines and diagnostics is complex, requiring many different skills. Each individual involved in a part of product R&D must be aware of the process overall and be able to relate their activities to it and to the needs of the other participating scientists and clinicians. Research scientists seek the discovery and confirmation of new knowledge by initiating or creating a hypothesis and then transforming it first into a theory and later into a new discovery. Product developers turn such discoveries into full-grown products which address public health needs through a long and quite different processes. Generally, research and development are two different disciplines. People working in these two areas do not think alike; they have different cultures. Often they work in isolation from each other, so they do not understand each other well. However, both disciplines are essential for the development of new drugs, vaccines and diagnostics. Furthermore, discovery of new knowledge is meaningless if it is not translated into new products that meet the needs of the public health.

Currently, there are only few courses in the north that give a good overview of the entire drug discovery and development process such as post-graduate courses at University of Cardiff, ECPM at University of Basel, University of Lyon and may be a few others. In majority of countries around the world, most of the topics related to product R&D are scattered throughout the various university curricula, including basic science, organic chemistry, immunology, pharmacy, pharmacology, vaccinology and clinical pharmacology. However, everyone involved in research or development must know their responsibilities and able to relate their tasks to all the others which make up the process of product R&D. The objective of the proposed course is to pull together the various components needed for product R &D into a dedicated MSc-PhD course. Discussions between different universities who are interested in this project have taken place on several occasions. Recently, six universities in four countries (Japan, Thailand, China and Colombia) have started working together to draft the content of the curriculum.

The curriculum is designed to provide basic knowledge of the product R&D process. It aims to demonstrate that new product discovery and the various development activities such as chemistry, toxicology, clinical investigations and regulatory practices are related as a continuous process, and that one discipline cannot carry out the whole process on its own.

Format of the course:

This is a joint project involving a number of universities in different countries. During the first five weeks, all students will take a core course on product R&D aimed at giving them an overview of the process. It will be held on a rotational basis in one of the participating universities. During the next ten weeks, students will select for in-depth exploration an area of their special interest, for example drug discovery, toxicology or clinical, and for this period will work in an institution that offers that specific area.

For the last 8 months, students will be attached to a specific institution, pharmaceutical company or biotech to work on a particular project.

The first four months of the course will be mainly lectures and case studies. The remaining 8 months will be mainly practical, including laboratory studies or clinical practice. The course is modular to allow those who already work in the area of product R&D to attend the appropriate parts of the course. This would provide such personnel with the opportunity of reviewing and discussing the special problems they encountered in their routine, real world work.

At the end of five weeks, students will be awarded a diploma in product research and development from Nagasaki University.

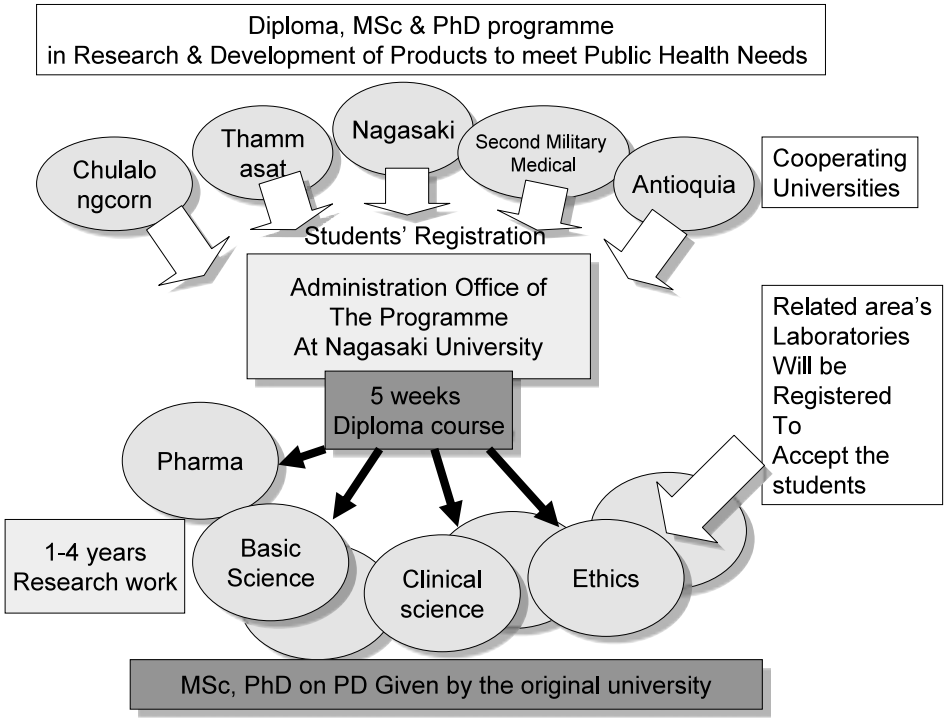
At the end of the first year, the successful students may receive a MSc. (subject to university requirement). Progression to PhD will depend on the evaluation of their first year performance. Those chosen as PhD candidates will do their thesis in the area of interest in one of the companies with on -going activities. Each student will receive their degree from the university that they registered for the course.

Contents of the core diploma course

This part of the course will begin with a general overview of product R&D (drugs, vaccines and diagnostics), continue with a series of lectures on discovery research, transitioning between research and development, CMC (chemical, manufacturing and control) requirements, toxicology requirements from development to product license, pharmaceutical and analytical development, pharmacokinetics and metabolic studies, clinical studies - phase I-IV trial design and protocols - project planning and management, handling of safety data in development, regulatory requirements, post regulatory clinical studies, patents issues and other aspects of product development i.e. international standards of good practice, ethics in clinical research, DSMB (Data and safety Monitoring Boards), commercial and marketing activities, and public health implementation.

Contents of the course during the next ten weeks:

The students will focus on the area of their interest. They will attend seminars and lectures in specific area such as discovery, CMC, toxicology, vaccinology, pharmacology, clinical development and regulatory requirements. For this, the student can choose to attend at their registered university (if such course is running during the period) or at another affiliated institution.



The program of core course in product R&D (Syllabus)

Module 1: Course Orientation

This module provides an over view of the need for a specific drug or vaccine for a disease or condition. The challenge for pharmaceutical research is to analyze a disease or condition to determine its effect on the body. This analysis leads to discovery of drug, vaccine or diagnostic which could then turn into a product. Resource requirements of current development paradigm and the time taken to develop a new product will be discussed.

The following topics will be covered:

- ○ Key medical and public health issues, and the need for new products
- ○ Discovery research and product development and the different approaches required for each of them
- ○ Resource implication for product development
- ○ Stakeholders in Product Research and Development
- ○ Major players
  - • Large, medium and small pharmaceutical companies
  - • Academic institutions
  - • CRO
  - • Biotechs
  - • Regulatory

Module 2: Drug Development

- ○ **Discovery Research:**

A comprehensive review of various approaches for new drug discovery from early history of mankind to contemporary techniques such as rational drug design, medicinal chemistry, in silico technology, genomics, proteomics, and pharmacogenomics will be covered. Examples of therapeutic areas derived from these approaches will be provided including lessons learned, pitfalls, and evolution towards a newer and more efficient approach. The importance of having the patents protected in the early stage as well as the strategy to publish (or not publish) the novel findings will be discussed.

The following topics will be covered:

- • Historical
- • Overview of modern drug discovery
- • Drug Targets
- • Lead Generation
- • Lead Optimization
- • Patents and publications

- ○ **Chemical Development: Chemistry**, **manufacturing and control (CMC):**

The required standards for composition, manufacture process, and controls information of the drug substance and the drug product that can ensure proper identification, quality, purity and strength of the investigating product will be described.

Following topics will be covered:

- Synthesis of active pharmaceutical ingredient
- Formulation
- Methods for determination of concentrations in various media by means of spectrometric methods, electromechanical methods, HPLC, gas chromatography and biological methods
- Stability for drug substance and drug product
- Development of specification
- Quality assurance/quality control
- Regulatory (with an example of a drug CMC requirement)
- Naming the New Chemical Entity

○ **Preclinical development**

The purpose of the Preclinical studies is to evaluate the pharmacological activity and toxicity of a drug candidate. The contents describe principles of pharmacology and toxicology, including the types of pharmacological and toxicological testing both *in vitro* and *in vivo* (animal model) and pharmacokinetics.

The following topics will be covered:

- Pharmacological development
  - Principles & knowledge of methodology
  - Pharmacological Tests:
    - √ *in vitro*
    - √ *Animal models*, *selection of suitable model and design*
- Toxicology
  - Principles of Toxicology
  - Toxicological Tests: *in vitro & in vivo:* acute, subacute, chronic, special organ toxicology, reproduction toxicology, teratogenicity, mutagenicity, carcinogenicity studies
  - Scheduling of toxicological studies in the development plan, the registration requirements, human & animal pharmacology, the proposed clinical application and the forms of administration.
  - Continuous monitoring of the correlation between new toxicological findings and the unwanted events observed in humans up till now.
- Pre-clinical Pharmacokinetics
  - Principles of Pharmacokinetics
    - √ ADME Processes
    - √ Pharmacokinetic Data Analysis & Pharmacokinetic Parameters
  - Transferability of the pharmacokinetic findings in animals to humans
- Investigating toxicological problems - practices and pitfalls
- Evaluation of viability (risk and benefit) for further development (case study)

○ **Clinical development**

The overview of clinical development will be presented, including the assessment of pre-clinical information package and clinical development plan. The important of human pharmacokinetics and pharmacodynamics in drug development will be discussed. Description of different clinical trial phases will be presented with examples. Different trial designs suitable for each type of studies will be illustrated, sample size calculation, statistical analysis plan and issues encountered during clinical development will be emphasized. An example of statistical analysis report will be demonstrated. Clinical data management methodology will be introduced. The regulatory requirements for clinical development and process of regulatory submission will be discussed.

Following topics will be covered:

- Overview
  - Assessment of pre-clinical information
  - Clinical development plan
  - Application of pharmacokinetics and pharmacodynamics in drug development
  - Dose selection and regimen
- Clinical trials
  - The various investigational phases of clinical research (Phases I-IV)
    - √ Human pharmacokinetics and pharmacogenetics
      - Definition and significance of pharmacokinetic parameters (absorption , bioavailability, binding to proteins, distribution, clearance, elimination half life, AUC)
      - Special human-pharmacokinetic studies e. g. bioavailability studies of multiple-dose, interaction studies, pregnancy, liver disease *etc*..
    - √ Therapeutic exploratory
    - √ Therapeutic confirmatory
    - √ Therapeutic use
    - √ Safety monitoring and reporting in clinical trials
  - Basic principles and evaluation of investigational results
    (Phase-I and early Phase-II), with a view to further development
  - Basic principles for decisions regarding further development or Discontinuation of a development project
- Study design

- possible study designs taking into account ethical aspects, indication, controls, patient population, location of the trial centers
- Trial design (parallel group design, cross over design, factorial design)
- Design techniques to avoid bias (blinding, randomization)
- Multi centers trials
- Type of comparison
- Group sequential designs
- Outcome measurements

- Statistical considerations
  - biostatistics in the planning phase (estimate of number of cases, randomization, statistical models, definition of end-points, planning of the subsequent evaluation)
  - Statistical analysis plan
  - Analysis sets: full analysis set, per protocol set, missing values and outliers
  - Data transformation
  - Method of statistical analysis (estimation, confidence intervals, hypothesis testing, evaluation of safety and tolerability
  - Statistical analysis report
- Introduction to Clinical Data Management
- Regulatory aspects of clinical development

Module 3: Vaccine Development

Rational: World eradication of small pox clearly showed that vaccine is a powerful tool to control the disease. First of all, we will discuss about the reasons why some vaccine developments were successful whereas some were not. Then we will discuss what types of vaccine or vaccine development are ideal to control the major diseases such as infectious diseases, cancer, autoimmune diseases etc.

○ **Discovery Research - Vaccines**

Vaccine effect is dependent on the acquired immunity that directed for the elimination of non-self including microorganisms, parasites, and cancer cells induced by the immunization of some appropriate antigens. What is the most effective acquired immunity against the disease? How do you select good antigens for such an effective vaccine development? And how do you make that antigen(s) more effective? We will see the most advanced knowledge and technology that will facilitate vaccine discovery research.

A historical review and overview of modern vaccine discovery will be presented. Various methods for antigen screening such as bioinformatics, protein chemistry, recombinant antigens, high-throughput, cell free recombinant system will be discussed. The evaluation of candidate antigens and selection of candidates for development will be described. Alternatives to using antigens will be presented. Examples of antigens derived from these approaches will be provided including lessons learnt, pitfalls, and evolution towards a newer and more efficient approach. Different adjuvant will be explored and discussed. The importance of having the patents protected in the early stage as well as the strategy to publish the novel findings will be discussed.

- Historical
- Overview of modern vaccine discovery
  - Understanding the basic immunology of the disease:
    Acquired immunity, Protective immunity, antigen, immunogenic
  - Biological Targets and Vaccine Candidates identification
  - Infectious disease, Cancer therapy, Autoimmune,
- Screening for antigens
  - Bioinformatics, Protein chemistry, recombinant antigens, high-throughput method including cell free recombinant system
- Evaluating antigens
  - In vitro and in vivo tests including animal model. Restriction for using animal model, genetically engineered animals
- Adjuvant
  - Type of the immune response provoked, new type of adjuvant, recombinant cytokine as adjuvant
- Alternatives to antigens
  - DNA vaccine, Live or attenuated pathogen
- Selection of development candidate and back-ups
  - Efficacy, toxicity, route if immunization, price, stability, cold chain,
- Patents and publications

○ **Antigen development**

After or during identification of vaccine antigens, it will be necessary to prepare appropriate amount and quality of antigens for further study. Process of scale-up, manufacture and control of the antigen will be demonstrated. Formulation of the vaccine antigen according to the required standard will be discussed.

- Scale-up, manufacture and control:
  - Types of large scale production of antigen, GLP and GMP
- Synthesis of antigen
  - Peptide, recombinant protein, DNA vaccine, recombinant BCG or organism, live or attenuated organism

- Synthesis of adjuvant
  - Mixture type or recombinant type
- Formulation
  - Soluble or suspension, Route, frequency, interval, number of dose, with or without adjuvant, mucosal immunization (aerosol, oral, nasal, inhalation, food), instability
- Quality assurance/quality control,
- Regulatory

○ **Preclinical development**

Preclinical Safety and immunogenicity assessment for vaccine development is performed by using animal model. There are two major check points, one is injection sites and the other systemic effect such as hypersensitivity and autoimmunity. Overview of the preclinical research will be demonstrated by using several typical experiences for vaccine development. Methods for the safety assessment and immunogenicity will be demonstrated. There will be a special session to demonstrate the classical and novel concept of animal model.

- Safety assessment

  Toxicity test for animal: regional complications, systemic toxicity such as fever, anaphylactic shock,
- Immunogenicity assessment
- Animal model used in pre-clinical studies
- The use of humanized animal model

○ **Clinical Development**

Clinical development of vaccine is somehow different from that of drug especially in the evaluation process and ethical aspects. The participants in vaccine trial are usually healthy volunteers and the sample size is normally greater than drug trial. It also takes longer duration to demonstrate the efficacy e.g. it needs a naturally infection to demonstrate the effect as challenging infection is not ethically acceptable. Administration of vaccine requires more complex procedures than drug trials, for example it requires cold chain, injection instruments, health workers, *etc*.

The assessment of pre-clinical information to proceed to human will be emphasized. In the Clinical trial section, the various investigational phases of clinical research (Phases I-IV) will be demonstrated. Basic principles and evaluation of investigation, development project, Study design, Statistical considerations, Data transformation Clinical Data Management, regulatory

- Overview
  - Assessment of pre-clinical information
  - Clinical development plan
  - Application of immunogenicity for vaccine development
  - Dose selection and regimen
- Clinical trial
  - The various investigational phases of clinical research (Phases I-IV)
    - √ Human immunogenicity and evaluation of efficacy
    - √ Confirmatory Studies
    - √ Vaccine use
    - √ Safety monitoring and reporting in clinical trials
  - Basic principles and evaluation of investigational results

    (Phase-I and early Phase-II), with a view to further development
  - Basic principles for decisions regarding further development or discontinuation of a development project
- Study design
  - possible study designs taking into account ethical aspects, indication, controls, population, location of the trial centers
  - Trial design (parallel group design, longitudinal design, factorial design, group sequential designs
  - Design techniques to avoid bias (blinding, randomization)
  - Multi centers trials
  - Type of comparison
  - Outcome measurements
- Statistical considerations
  - biostatistics in the planning phase (estimate of number of cases, randomization, statistical models, definition of end-points, planning of the subsequent evaluation)
  - Statistical analysis plan
  - Analysis sets: full analysis set, per protocol set, missing values and outliers
  - Data transformation
  - Method of statistical analysis (estimation, confidence intervals, hypothesis testing, evaluation of safety and tolerability
  - Statistical analysis report
- Introduction to Clinical Data Management
- Regulatory

Module 4: Diagnostic Development

Diagnostic tools in combination with therapeutic or preventive medical care are important to develop for public health purpose. Without good diagnostic method, it would be impossible to evaluate the disease burden in the community, to

treat the patients and to protect the society against the disease.

Practical approach towards the development of really effective diagnostic tools for public health will be demonstrated and discussed. In the overview, several excellent examples will be shown. Discovery session will show three steps for the discovery, Necessity assessment, technology selection, prototype production. Evaluation of clinical applicability such as Sensitivity and specificity will be discussed. Detailed protocol for the Clinical development will be demonstrated.

- Overview
- Discovery and Development of diagnostic tools
  - Necessity assessment, Principles and technology selection, prototype production and assessment.
    - Identify preliminary diagnostic test
    - Validation of clinical potential
    - Identification of new targets using genomics and protein and cellular studies
    - Development of potential technology platform
    - Principles of diagnostic methods antibody detection, antigen detection, biological parameters including DNA, RNA, enzymes, proteins
    - Define product specifications
    - Feasibility assessment
- Scale-up, manufacture and control:
  - Practical Application:

  Development of kits, necessary equipments, electricity, technician, budget
  - Quality assurance/quality control: Evaluation of the efficacy after application
- Clinical Development:
  - Validate prototype
  - Manufacture pilot lot
  - Initiate clinical trials
  - Supply chain logistics and production
  - Implementation
  - Statistical considerations
  - Regulatory matters

Module 5: Standards in Clinical Research and Development

Regulations and guidelines vary from one country to another. These regulations and guidelines dictate on how to develop the product in each country. The product developer must meet all the requirements and expectations of the regulatory authorities as efficiently as possible. The module describes the guidelines and regulatory requirements in various countries.

- Good Manufacturing Practice:
- Good Laboratory Practice:
- Good Clinical Practice:
  - ICH
  - WHO
- Ethics Codes and Guidelines:
  - Declaration of Helsinki
  - CIOMS
  - Belmont Report
  - WHO Operational Guidelines for Ethics Committees that Review Biomedical Research
- Principles of Research Ethics Autonomy, Beneficence, Justice
- Research Methodologies and Ethical Issues

  Biomedical Research including traditional medicine

  In Various Types of Health Research Genetic Research & Stored Samples
- Ethics Committees
- Data and Safety Monitoring Boards
- Clinical Data Management
  - Data protection aspects
- Clinical study monitoring
- Audits and inspections
- US FDA Guidelines and regulation
- EMEA Guidelines and regulations
- Japan Guidelines and regulations
- China Guidelines and regulations
- Thailand Guidelines and regulations
- Colombia Guidelines and regulations

Module 6: Clinical Data Management

Project planning and management at every stage of development will be described. Developmental objectives, crucial milestones, concise detailed analysis of product and roadmap to market will be demonstrated, including Patent process. Regulatory strategies and strategies for dealing with potential roadblocks and hurdles in the product development process will be discussed. The plan will include a lay out of an accurate and realistic budgets and timelines throughout the project development. A practical workshop on project planning and management will be included. Following topics will be covered:

- Project planning and management, including practical session
- Data acquisition
- Data Privacy
- Data Capture Principles
  - CRF Design & Completion Guidelines
  - Electronic Data Capture
- Database

- Data Storage
- Database Validation
- Database Programming and Standards

- Data
  - Data Entry
  - Data Processing: Validation (Edit Check Specification)
  - Laboratory & External Data
- Dictionary Management
  - Adverse Events
  - Drug
- Reporting
  - Safety Data Management and Reporting

Module 7: Activities after Registration

Objective:

a. to identify stakeholders to be involved in post regulatory activities, their functions and roles in bringing the products to solve the intended public health problems.

b. describe the policy instruments to bring the products to the intended beneficiaries.

c. develop strategies for public and private partnership to encourage

- Research and development in areas of need and
- Advocate the public sector to allocate funds to allocate funds to bring products to the intended beneficiaries.

d. develop evidence based actionable message, identify resources requirement to scale up the products for use in the health care system (diagnostic tests, vaccines and drugs) as well as describe strategies to mobilize these resources.

e. describe mechanisms and strategies for post marketing product vigilance for product quality, post marketing efficacy and side effects.

f. Identify the human resource capacity strengthening needs and strategies to fulfil those needs such as through best practice health services research using "unqualified" personnel and training of the trainers.

○ **Stakeholders to be involved in making product development work for the intended beneficiaries**

Stakeholders in health care systems are important in making development products work for poor people and intended beneficiaries. These include the policy makers, the system managers, directors of facilities, the practitioners and the intended beneficiaries. Each of these stakeholders has unique responsibility, roles and functions. The roles and functions have to be coordinated to make the system make the products work. The unique roles, functions and the tools to coordinate the stakeholders to make the product work will be described with specific examples for HIV/TB care and addressing poverty gender based inequalities and how to deal with them. Specific stakeholders to be discussed in details are:

- Public policy
- System policy
- Facility Policy
- Practice Policy
- Empowerment of public

○ **Policy Instrument**

Social factors to a large extent shape the success for failure of bring innovative products to benefit the intended beneficiaries. Policy instruments are needed to deal with the complexity of social impediments to health and diseases. The use of the policy instruments will need the right understanding of diseases and their proximate and distant determinants. The right understanding can give insight to: a) targeting the products; b) development rules and regulation to procure and provide the products to the intended beneficiaries; c) allocation of resource to finance the purchase of the products to target beneficiaries and d) development of services either at the public or private sectors where appropriate. Specific instruments to be discussed in details are:

- Public health need and vulnerable groups
- Targeting
- Rule regulation (financing, guidelines)
- Resources allocation
- Service planning: primary, secondary, tertiary care

○ **Public Private Partnership (3 hours)**

The purpose of partnership between the public and private sector is to promote the interface between product development and their use in clinical and systems settings. Partnership can have an effect of the overall priorities and successes of product development. A good partnership will strengthen the credibility of the relationship between the public and private sector over the long term. There are several possible reasons to develop a public and private partnership. The most important one should be the need to achieve a task, which is not achievable if each of the partners works independently. Typically, these activities help control a 'neglected' disease or condition in developing countries such as through development or distribution of a drug, diagnostics, vaccines, contraceptive and other products. In general, there are two types of partnership:

1) those that want to tackle a problem in a more efficient way; and

2) those that are created to tackle what is conceived as intractable problems such as the development of a malaria vaccine. These partnerships want to find new ways of tackling the problems because the world does not yet know how to do it. Since the cost of product development can be high, economic consideration to promote an interface between development/clinical use and approval, post marketing must be in place. Both the "push" and "pull" mechanisms will be described. The "push" mechanism guide the research and development initiatives, while the "pull" mechanism ensure that the public sector will guarantee allocation of funds to purchase products for the intended beneficiaries once they are available.

- Public-private partnership
- Function and structure of partnerships
- Good characteristic of partnerships
- Monitoring partnerships
- Examples of partnerships for product research and development

○ **Improving the quality use of new products in health systems**

Evidence for efficacy and safety of the products must be interpreted for potential users of the products to enhance quality use for intended beneficiaries. The potential users include policy makers, practitioners, patients and public, including the media. The interpretations have to be transformed into evidence based actionable products relevant for each of potential users. Other strategies such as the "triangle that moves the mountain" and the "academic NGO" movement of the University of Ottawa can be used to develop strategies to link evidence based actionable message to potential users.

- **Diagnosis**
  - Characteristics of tests and resource needed to implement the test in health system.
  - Access to diagnostic services and case finding for poverty and gender-based inequalities
  - Balancing public protection and stigmatization and denial (TD/HIV)
- **Vaccine:**
  - Characteristics of Vaccine and resource needed to implement the test in health system.
  - Coverage and herd immunity
  - Post vaccination exposure and risk activities
- **DRUG**.
  - Indications, contraindications, use and resource needed to implement the test in health system.
  - Compliance of provider
  - Compliance of subjects
  - Measures to improve compliance

○ **Post marketing product vigilance**

New products, such as drugs, vaccines and diagnostic tests have both benefits and side effects, some of which might not be apparent until the products have been used over a long period of time. Therefore, a system must be developed to measure the benefits as well as risks. Benefits need to be weighed against the occurrence of adverse events. A risk/benefit analysis of the products must be evaluated using standardized tools and procedures. The importance of guidance for standardization of terminology, data collection, verification and presentation of efficacy and adverse reaction reports will be emphasized and discussed.

Possible topics include:

- The definition of pharmacovigilance.
- The scope, instruments/tools, and processes needed for Pharmacovigilance of medicinal products for human use.
- Systems for standardization of pharmacovigilance reporting and exchange of pharmacovigilance information and subsequent appropriate actions.
- Administrative and legislative information relevant to medicinal products for human use.
- The mechanism for reviewing and updating legislative and technical areas for general use.

○ **Capacity for optimal delivery of new product: Training and Health services research**

It is important that countries have the capacity to identify, innovate and adapt new products to its own need and constraints in order to address their unique burden of illnesses including the burden of tropical diseases. At times, health service research might be carried out to document the possibility of using products via "unqualified personnel" through training to ensure best practice (such as the use of nurses for provide contraceptive services). Policy formulation, implementation and evaluation must be in place. Most developing countries do not have capacity to formulate policy identify, innovate and adapt new products to relevant to their problems. Vaccines against ROTA virus, which was not approved in the US due to rate intussusceptions might have be very beneficial in developing countries in preventing burden of illnesses from ROTA virus diarrhea over the incidence of complications because the incidence of ROTA virus diarrhea is high. Likewise, capacities for the development of treatment guidelines and their financing, and optimal facility planning for new products are needed to optimally distribute and use the new products for the intended beneficiaries. Individual practitioners also need skills to search for the best evidence about the use of products within the constraints of their health systems. Various models of international collaboration for capacity strengthening are available

such as the Thai Golden Jubilee grant. The topics to be considered and discussed include:

- Policy formulation, implementation and evaluation
- Guidelines & Finance (insurance)
- Optimal facility planning and program management.
- Evidence based search for best information for practitioners, subjects
- Model for capacity strengthening through international collaboration.

Agenda of the diploma course of 2006

Diploma Course on Research & Development of Products to Meet Public Health Needs

Sponsored by Nagasaki University
in cooperation with Thammasat University, Chulalongkorn University, China Second Military Medical University, Antioquia University and The Graduate School of Pharmaceutical Sciences of The University of Tokyo
in collaboration with WHO and The Pharmaceutical Society of Japan (PSJ)

Nagasaki University, Japan
October 2—November 8, 2006

Tentative agenda

Module 1 Course Orientation

October 2, 2006 Monday

0900-0915 Welcome address
President, Prof Dr. Hiroshi Saitoh, Nagasaki University, Japan

0915-0945 Objective of the course
Professor Dr. Kenji Hirayama, Director of the course, Nagasaki University, Japan

0945-1000 Introductions of participants

1000-1030 *Tea break*

1030-1200 Key medical and public health issues, and the need for new products
Dr. Janis Lazdins, WHO/TDR, Geneva

1200-1300 *Lunch*

1300-1400 Discovery research and product development and the different approaches required for each of them
Dr. Janis Lazdins, WHO/TDR, Geneva

1400-1500 Stakeholders in Product Research and Development
- Large, medium and small pharmaceutical companies
- Academic institutions
- Clinical Research Organization
- Biotech
- Regulatory

Prof. Dr. Eiji Uchida, Showa University, Tokyo, Japan

1500-1530 *Tea break*

1530-1600 Stakeholders in Product Research and Development

1600-1630 Q&A

Module 2 Drug Development
Drug Discovery

October 3, 2006 Tuesday

0900-11.00 History and overview of modern drug discovery
Mr Nobuhiro Noro, GSK, Japan

1100-1130 *Tea Break*

1130-1230 From drug target to drug lead
Mr Nobuhiro Noro, GSK, Japan

1230-1330 *Lunch*

1330-1430 Drug targets identification and validation in malaria
TBA

1430-1530 Drug targets identification and validation in TB
Assoc. Prof. Dr. Prasit Palithapolkarnpim, BIOTEC, Thailand

1530-1600 *Tea break*

1600-1700 Drug targets identification and validation in cardiovascular diseases
Dr.Kihito Takahashi, Japanese Association of Pharmaceutical

Medicine (JAPHMED), Merck Banyu Pharma, Japan

October 4, 2006 Wednesday

0900-1030 Overview of chemistry in drug discovery
Hit/lead generation and optimisation
Dr. Prof. Yoshimoto, Nagasaki University, Nagasaki, Japan

1030-1100 *Tea break*

1100-1200 Drug discovery for Prion disease
Prof. Shigeru Katamine, Nagasaki University, Nagasaki, Japan

1200-1300 *Lunch*

1300-1430 Visit laboratory in Nagasaki University (Prion Lab)

1430-1530 Drug discovery for TB
TBA

1530-1600 *Tea break*

1600-1700 Drug discovery for Trypanosomiasis
Prof. Dr. Kiyoshi Kita, University of Tokyo, Japan

October 5, 2006 Thursday

0900-1000 Publications, IPR and patents in drug discovery
Mr. Kenichi Osawa, Merck Banyu Pharma, Japan

1000-1030 *Tea break*

1030-1130 Publications, IPR and patents in drug discovery (Cont.)
Mr. Kenichi Osawa, Merck Banyu Pharma, Japan

Chemical Manufacturing and Control (CMC)

October 6, 2006 Friday

0900-1000 Synthesis of active pharmaceutical ingredient
Prof Susumi Hatakeyama, Nagasaki University, Japan

10:00-10:30 Formulation
Prof Susumi Hatakeyama, Nagasaki University, Japan

1030-1100 *Tea break*

1100-1300 Methods for determination of concentrations in various media by
means of spectrometric methods, HPLC, and biological methods
Prof.Dr. Masaaki Kai, Nagasaki University, Japan

1300-1400 *Lunch*

1400-1530 Stability for drug substance and drug product
Prof.Dr. Hiroaki Nagaoka, Nagasaki International University, Japan

1530-1600 *Tea break*

1600-1700 Development of specification
Prof.Dr. Hiroaki Nagaoka, Nagasaki International University, Japan

October 7, 2006 Saturday

0900-1030 Quality assurance/quality control
Prof.Dr. Hiroaki Nagaoka, Nagasaki International University, Japan

1030-1100 *Tea break*

1100-1200 Example: Antimalarial drug, dihydroartemisinin
Assoc. Prof. Supornchai, Mahidol University, Thailand

1200-1300 *Lunch*

1300-1530 Regulatory (with an example of a drug CMC requirement)
Prof.Dr. Hiroaki Nagaoka, Nagasaki International University, Japan

1530-1600 *Tea break*

1600-1630 Naming the New Chemical Entity (NCE)
Prof.Dr. Hiroaki Nagaoka, Nagasaki International University, Japan

Pre-clinical Development

Pharmacological development

9 October 2006 Monday

0900-1100 Pharmacological data in new drug application
Dr. Shunsuke Ono, University of Tokyo, Japan

1100-1130 *Tea break*

1130-1230 Methods in pharmacological R&D (1)
Dr. Hiroyuki Itoh, Astellas Pharma Inc, Japan

1230-1330 *Lunch*

1330-1430 Methods in pharmacological R&D (2)
Dr. Hiroyuki Itoh, Astellas Pharma Inc, Japan

1430-1500 Discussion
Drs. Shunsuke Ono and Hiroyuki Itou

1500-1530 Tea break

1530-1630 The cure oriented therapeutics for chronic renal failure with gene therapy
Dr. Tsutomu Kurosawa, Osaka University, Japan

Toxicology

10 October 2006 Tuesday

0900-1000 Principles of toxicology
Assoc. Prof.Dr. Wongwiwat Tassaneeyakul, Kon Kaen University, Thailand

1000-1100 Toxicological tests: *in vitro & in vivo:* acute, subacute, chronic, special organ toxicology, reproduction toxicology, teratogenicity, mutagenicity, carcinogenicity studies
Assoc. Prof.Dr. Wongwat Tassaneeyakul, Kon Kaen University, Thailand

1100-1130 *Tea break*

1130-1300 Scheduling of toxicological studies in the development plan, the registration requirements, human & animal pharmacology, the proposed clinical application and the forms of administration.
Dr. Soisanwan Satarug, University of Queensland, Australia

1300-1400 *Lunch*

1400-1530 Continuous monitoring of the correlation between new toxicological findings and the unwanted events observed in humans up till now.
Dr. Soisanwan Satarug, University of Queensland, Australia

1530-1600 *Tea break*

Pre-clinical Pharmacokinetics

11 October 2006 Wednesday

0900-1030 Principles of pharmacokinetics: ADME processes
Assoc. Prof.Dr. Wongwat Tassaneeyakul, Kon Kaen University, Thailand

1030-1100 *Tea break*

1100-1230 Pharmacokinetic data analysis & pharmacokinetic parameters
Assoc. Prof.Dr. Wongwat Tassaneeyakul, Kon Kaen University, Thailand

1230-1330 *Lunch break*

13:30-1530 Transferability of the pharmacokinetic findings in animals to humans investigating toxicological problems - practices and pitfalls
Dr. Soisanwan Satarug, University of Queensland, Australia

12 October 2006 Thursday

1000-1200 Visit animal facility for medical research (Sato animal house)

1500-1630 Evaluation of viability (risk and benefit) for further development
(Case study)
Dr Tadaaki Taniguchi, Japanese Association of Pharmaceutical Medicine (JAPHMED), Merck Banyu Pharma, Japan

Clinical Development

Clinical Trial

13 October 2006 Friday

0900-1100 Overview of clinical development
- Assessment of pre-clinical information
- Clinical development plan
- Application of pharmacokinetics and pharmacodynamics in drug development

Dose selection and regimen
Dr. Tadaaki Taniguchi, Japanese Association of Pharmaceutical Medicine (JAPHMED), Merck Banyu Pharma, Japan n

1100-1130 *Tea break*

1130-1200 The various investigational phases of clinical research (Phases I-IV)
Dr. Tadaaki Taniguchi, Japanese Association of Pharmaceutical Medicine (JAPHMED), Merck Banyu Pharma, Japan

1200-1300 *Lunch*

1300-1500 Human pharmacokinetics:
- Definition and significance of pharmacokinetic parameters (absorption , bioavailability, binding to proteins, distribution, clearance, elimination half life, AUC)
- Special human-pharmacokinetic studies e. g. bioavailability studies of multiple-dose, interaction studies, pregnancy, liver disease *etc*.

Prof. Dr. Kesara Na Bangchang, Director, Graduate Program in Biomedical Sciences, Thammasat University, University, Thailand

1500-1530 *Tea break*

14 October 2006 Saturday

0900-1000 Therapeutic exploratory (with example)
Dr. Kenji Nonaka, Japanese Association of Pharmaceutical Medicine (JAPHMED), Merck Banyu Pharma, Japan

1000-1100 Therapeutic confirmatory (with example)
Dr. Kenji Nonaka, Japanese Association of Pharmaceutical Medicine (JAPHMED), Merck Banyu Pharma, Japan Tea break

1100-1130 *Tea Break*

1130-1230 Therapeutic use (with example)
Dr.Kimihiro Kasamo, Japanese Association

of Pharmaceutical Medicine (JAPHMED), Merck Banyu Pharma, Japan

1230-1330 *Lunch*

1330-1500 Safety monitoring and reporting in clinical trials
- Basic principles and evaluation of investigational results (Phase-I and early Phase-II), with a view to further Development
- Basic principles for decisions regarding further development or discontinuation of a development project

Dr.Kimihiro Kasamo, Japanese Association of Pharmaceutical Medicine (JAPHMED), Merck Banyu Pharma, Japan Tea break

1500-1530 *Tea Break*

1530-1630 Pharmacogenomics

Dr. Shyh-Yuh Liou, Japan Section GlaxoSmithKline, Japan

Study design

16 October 2006 Monday

0900-1030 Study design
- Possible study designs taking into account ethical aspects, indication, controls, patient population, location of the trial centers
- Trial design (parallel group design, cross over design, factorial design, group sequential design)
- Design techniques to avoid bias (blinding, randomization)

Prof. Dr. L. Jeeyaseelan, Christian Medical University, Vellor, India

1030-1100 *Tea break*

1100-1230 Study design (Cont.)
- Multi centers trials
- Type of comparison
- Outcome measurements

Prof .Dr. L. Jeeyaseelan, Christian Medical University, Vellor, India

1230-1330 *Lunch*

1330-1500 Statistical considerations
- Biostatistics in the planning phase (estimate of number of cases, randomization, statistical models, definition of end-points, planning of the subsequent evaluation)
- Statistical analysis plan
- Analysis sets: full analysis set, per protocol set, missing values and outliers

Prof. Dr. L. Jeeyaseelan, Christian Medical University, Vellor, India

1500-1530 *Tea break*

1530-1700 Statistical considerations (Cont.)
- Data transformation
- Method of statistical analysis (estimation, confidence intervals, hypothesis testing, evaluation of safety and tolerability)
- Statistical analysis report

Prof. Dr. L. Jeeyaseelan, Christian Medical University, Vellor, India

Regulatory Issues

17 October 2006 Tuesday

0900-1030 Regulatory aspects of clinical development

Dr. Kazuhiko Mori, Office of New Drug1, Center for Product Evaluation, Pharmaceutical and Medical Devices, PMDA, Japan

1030-1100 *Tea break*

1100-1230 Special topics:
- Genetic engineer product
- Gene therapy and stem cells

Dr. Kazuhiko Mori, Office of New Drug1, Center for Product Evaluation, Pharmaceutical and Medical Devices, PMDA, Japan

1230-1330 *Lunch*

1530-1600 Example of Clinical Drug development in Inida - Miltefosine trial

Prof. Dr. Juntra Karbwang, WHO/TDR, Switzerland

Traditional Medicine

18 October 2006 Wednesday

0900-1030 Introduction to traditional Medicine: drug discovery and development

Professor Dr. Kiichiro Tsutani, University of Tokyo, Japan

1030-1100 *Tea break*

1100-1200 Guidance on herbal medicine

Prof. Dr. Juntra Karbwang, WHO/TDR, Switzerland

1300-1500 Regulation for traditional medicine development

Japan: *Dr. Ichiro Arai, Manager, R&D Strategy Dept. Tsumura & Co.*

China: *Dr. Luping Qin, China*

Thailand: *Dr. Vichai Chokevivat, Director, Department of Alternative Medicine, MOH Thailand*

1500-1530 *Tea break*
1530-1700 Example: Herbal medicine to modern medicine
Example: traditional medicine development
Dr. Luping Qin, China

Module 3: Vaccine Development
Vaccine Discovery

19 October 2006 Thursday
0900-0930 Historical of vaccine Discovery
Dr. Howard Engers, AHARI, Ethiopia
0930-1030 Overview of modern vaccine discovery
Dr. Howard Engers, AHARI, Ethiopia
1030-1100 *Tea break*
1100-1200 Screening for antigens
Prof Dr. Kenji Hirayama, Nagasaki University, Japan
1200-1330 *Lunch*
1330-1430 Evaluating antigens
Prof Dr. Kenji Hirayama, Nagasaki University, Japan
1430-1500 *Tea break*
1500- Visiting Vaccine Discovery Laboratory Institute of Tropical Medicine, Nagasaki University

20 October 2006 Friday
0900-1030 Adjuvant -
Dr. Howard Engers, AHARI, Ethiopia
1030-1100 *Tea break*
1100-1200 Alternatives to antigens: DNA vaccine, Live or attenuated pathogen
Dr. Howard Engers, AHARI, Ethiopia
1200-1330 *Lunch*
1330-1430 Selection of development candidate and back -ups
Dr. Howard Engers, AHARI, Ethiopia
1430-1500 *Tea break*
1500-1630 Efficacy, toxicity, route if immunization, price, stability, cold chain,
Dr. Howard Engers, AHARI, Ethiopia

21 October 2006 Saturday
0900-1030 Malaria vaccine discovery
Prof. Dr.Weiqing Pan, China
1030-1100 *Tea break*
1100-1200 Cholera vaccine discovery
Dr. Masahiko Ehara, Nagasaki University, Japan
1200-1330 *Lunch*
1300-1400 West Nile Fever vaccine discovery
Prof. Dr.Kouichi Morita, Nagasaki University, Japan
1400-1500 Oral vaccine discovery
Dr. Takeshi Arakawa, Ryukyu University, Japan
1500-1530 *Tea break*

Antigen Development
23 October 2006 Monday
0900-1030 Scale-up, manufacture and control:
Types of large scale production of antigen, GLP and GMP
Dr. Horiuchi, GlaxoSmithKline, Tokyo, Japan
1030-1100 *Tea break*
1130-1230 Synthesis of antigen
Peptide, recombinant protein, DNA vaccine, recombinant BCG or organism, live or attenuated organism
Dr. Horiuchi, GlaxoSmithKline, Tokyo, Japan
1230-1330 *Lunch*
1330-1430 Synthesis of adjuvant
Mixture type or recombinant type
Dr. Horiuchi, GlaxoSmithKline, Tokyo, Japan
1430-1500 *Tea break*
1500-1530 Formulation
Soluble or suspension, Route, frequency, interval, number of dose, with or without adjuvant, mucosal immunization (aerosol, oral, nasal, inhalation, food), instability
Dr. Horiuchi, GlaxoSmithKline, Tokyo, Japan
1530-1600 Quality assurance/quality control
Dr. Horiuchi, GlaxoSmithKline, Tokyo, Japan

Clinical Development
24 October 2006 Tuesday
0900-0930 The various investigational phases of clinical research (Phases I-IV)
Dr. Horiuchi, GlaxoSmithKline, Tokyo, Japan
0930-1030 Basic principles and evaluation of investigational results
- Phase-I and early Phase-II, with a view to further development

Dr. Horiuchi, GlaxoSmithKline, Tokyo, Japan

1030-1100 *Tea break*

1100-1230 Basic principles for decisions regarding further development or discontinuation of a development project
Dr. Horiuchi, GlaxoSmithKline, Tokyo, Japan

Pre-Clinical Development

1330-1500 The use of humanized animal model
Dr. Kenji Hirayama, Nagasaki University, Japan

25 October 2006 Wednesday

0900-1030 Animal model used in pre-clinical studies
Dr. Shigeyuki Kano, International Medical Center of Japan, Tokyo

1030-1100 *Tea break*

Clinical Development

Overview

1100-1130 Assessment of pre-clinical information
TBA

1130-1230 Clinical development plan
Professor Dr. Kenji Hirayama, Nagasaki

1230-1330 *Lunch*

1330-1430 Application of immunogenicity for vaccine development
Dr. Shigeharu Ueda, The Research Foundation for Microbial Diseases of Osaka University (BIKEN), Japan

1430-1500 *Tea break*

1500-1600 Dose selection and regimen
Dr. Shigeharu Ueda, The Research Foundation for Microbial Diseases of Osaka University (BIKEN), Japan

Pre-Clinical Development

26 October 2006 Thursday

0900-1030 Safety assessment
Toxicity test for animal: regional complications, systemic toxicity such as fever, anaphylactic shock
Mr. Nobuhiro Noro, GlaxoSmithKline, Tokyo, Japan

1030-1100 *Tea break*

1100-1230 Immunogenicity assessment
Mr. Nobuhiro Noro, GlaxoSmithKline, Tokyo, Japan

1230-1330 *Lunch*

1330-1430 Regulatory
Mr. Yoshino, Dr. Masaru Iwasaki, GlaxoSmithKline, Tokyo, Japan

1430-1500 *Tea break*

1500-1600 Example: Malaria Vaccine Clinical Trial Development
TBA

1600-1700 Example: TB Vaccine Clinical Trial
TBA

Module 4: Diagnostic Development

27 October, 2006 Friday

0900-1030 Discovery and development of diagnostic tools:
Necessity assessment, Principles and technology selection
Dr.Masato Sasaki, Roche Diagnostics KK, Tokyo, Japan

1030-1100 *Tea break*

1100-1230 Prototype production and assessment
Dr.Masato Sasaki, Roche Diagnostics KK, Tokyo, Japan

1230-1400 *Lunch*

1400-1530 Scale-up, manufacture and control
Dr. Masato Sasaki, Roche Diagnostics KK, Tokyo, Japan

1530-1600 *Tea break*

1600-1730 Scale-up, manufacture and control (Cont.)

28 October, 2006 Saturday

900-1030 Development of kits
Dr. Masato Sasaki, Roche Diagnostics KK, Tokyo, Japan

1030-1100 *Tea break*

1100-1230 Quality assurance/quality control: evaluation of efficacy after application
Dr. Masato Sasaki, Roche Diagnostics KK, Tokyo, Japan

1230-1400 *Lunch*

1400-1530 Clinical development: validate prototype, manufacture pilot lot, and initiate clinical trial
Dr. Masato Sasaki, Roche Diagnostics KK, Tokyo, Japan

1530-1600 *Tea break*

1600-1730 Clinical development: Supply chain logistics and production, Statistical consideration, regulatory issues
Dr. Masato Sasaki, Roche Diagnostics KK, Tokyo, Japan

Module 5: Standards in Clinical Research and Development

Ethics in research and Ethics Committee

30 October, 2006 Monday

0900-1000 Ethics Codes and Guidelines
Prof. Dr. Cristina Torres, FERCAP, Thailand

1000-1100 Principles of Research Ethics
Prof. Dr. Cristina Torres, FERCAP, Thailand

1100-1130 *Tea break*

1130-1230 Case study

1230-1400 *Lunch*

1400-1500 Research methodology and ethical issues (1) Traditional medicine
Dr. Vichai Chokevivat, Director, Department of Alternative Medicine, MOH Thailand

1500-1600 Research methodology and ethical issues (2) Genetic study
Prof. Dr. Kenji Hirayama, Nagasaki University, Japan

1600-1630 *Tea break*

1630-1730 Case study

31 October, 2006 Tuesday

0900-1030 Ethics Committee
Prof. Dr. Cristina Torres, FERCAP, Thailand

1030-1100 *Tea break*

1100-1230 Ethics committee Cont.
Prof. Dr. Cristina Torres, FERCAP, Thailand

1230-1400 *Lunch*

1400-1500 Data and Safety Monitoring Board (DSMB)
Prof. Dr. Juntra Karbwang, WHO/TDR, Switzerland

1500-1630 Case study

1630-1730 Monitoring and auditing Ethics Committee
Prof. Dr. Cristina Torres, FERCAP, Thailand

1 November, 2006 Wednesday

Quality Standards

0900-09.30 Concept of Good Clinical Practice
Dr. Johansen, Allan, Roche Products Pty limited, Australia

09.30-11.30 Responsibilities
Sponsor (*Dr. Allan Johansen*)
Investigators (*Prof. Kenji Hirayama*)
IRB (*Prof. Cristina Torres*)
Monitors (*Prof. Juntra Karbwang*)
DSMB (*Dr. Allan Johansen*)

11.30-12.00 Audit and Inspection
Dr. Johansen, Allan, Roche Products Pty limited, Australia

1200-1300 *Lunch*

14:00-15:30 New Asymmetric Catalysis; Leading to the synthesis of Tamiflu
Prof. Dr. Masakatsu Shibasaki, The University of Tokyo, Japan

2 November, 2006 Thursday

In the morning: Field Trip to Kaketsuken, Kumamoto by Bus:

13:00-17:00 Good Manufacturing Practice (GMP)
Good Laboratory Practice (GLP)
Dr. Kyousuke Mizuno, Kaketsuken, Kumamoto, Japan
Visit GMP lab and GLP lab and Plant for vaccine production

3 November, 2006 Friday Holiday

Module 6: Clinical Data Management

6 November, 2006 Wednesday

0900-1000 Overview of clinical data management
Data management plan
Dr. Charcrin Na-Bangchang, TU-CDMC, Thailand

1000-1030 Statistical Analysis Plan (SAP)
Data: primary & secondary data
Dr. Rui Wang, SMMC-CDMC, China

1030-1100 *Tea break*

1100-1230 Data capture , development of database
Prof. Dr. L. Jeeyaseelan, CMC-CDMC, India

1230-1330 *Lunch*

1330-1400 Data entry, data verification, data validation, audit trail
Data clarification process
Data query and resolution
Prof. Dr. Kenji Hirayama, CMC-CDMC, Nagasaki University, Japan
Dr. Lawrence Yamua, AA-CDMC, Ethiopia

1400-1500 Data transform process
- Adverse Event Dictionary
- Drug Dictionary

Dr. Sangkae Chamnanawakit, TU-CDMC, Thailand

1500-1530 *Tea break*

1530-1700 Statistical analysis
Dr. Arunachalam Rajapopal, CMC-CDMC, India

1700-1800 Quality Control & Assurance (QC & QA)
Standard Operating Procedures (SOPs)
Dr. Jose Fernando Florez Arango, CMC-CDMC, Colombia

Module7: Post-registration Activities

7 November 2006 Tuesday

0900-1000	Stakeholders to be involved in making product development work for the intended beneficiaries *Prof. Dr. Chitr Sitthi-amorn, Chulalongkorn University, Thailand* *Prof. Dr. Pakdee Pothisiri, FDA, Thailand* *Prof. Dr. Kazuko Kimura, Kanazawa University, Japan* *Dr.Kihito Takahashi, Japanese Association of Pharmaceutical* *Medicine (JAPHMED), Merck Banyu Pharma, Japan*
1000-1100	Policy Instrument *Prof. Dr. Pakdee Pothisiri, FDA, Thailand* *Prof. Dr. Chitr Sitthi-amorn, Chulalongkorn University, Thailand*
1100-1130	*Tea break*
1130-1230	Public private partnership *Prof. Dr. Chitr Sitthi-amorn, Chulalongkorn University, Thailand* *Prof. Dr. Pakdee Pothisiri, FDA, Thailand* *Prof. Dr. Kazuko Kimura, Kanazawa University, Japan*
1230-1330	*Lunch*
1330-1430	Public private partnership Cont.
1430-1500	*Tea break*
1500-1700	Pharmacoeconomics *Prof. Dr. Kiichiro Tsutani, University of Tokyo, Japan*

8 November 2006 Wednesday

0900-1700	Improving the quality of new products in health systems: International network of regional use of drugs *Prof. Dr. Chitr Sitthi-amorn (Chulalongkorn University)*
0900-1030	Post-marketing product vigilance *Dr. Janis Lazdins, TDR/WHO, Geneva, Switzerland* *Prof. Dr. Chitr Sitthi-amorn, Chulalongkorn University, Thailand* *Prof. Dr. Pakdee Pothisiri, FDA, Thailand*
1100-1130	*Tea break*
1100-1200	Capacities for optimal delivery of new products: training and health service research *Prof. Dr. Chitr Sitthi-amorn, Chulalongkorn University, Thailand*
1200-1300	*Lunch*
1300-1430	Intellectual Property Rights Protection in Developing Countries *Prof. Dr. Hiroko Yamane, Graduate Institute for Policy Studies, Japan*
1430-1500	*Tea break*
1500-1630	Product life cycle *Dr. Janis Lazdins, TDR/WHO, Geneva, Switzerland*

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BEHAVIORS ASSOCIATED WITH WATER CONTACT AND *SCHISTOSOMA JAPONICUM* INFECTION IN A RURAL VILLAGE, THE DONGTING LAKE REGION, CHINA

Shouhei Takeuchi[1,2], Yuesheng Li[3], Yongkang He[3], Huan Zhou[1],
Kazuhiko Moji[2], Ryutaro Ohtsuka[4], Chiho Watanabe[1]
Accepted 29, June, 2006

Abstract: Although identification of water contact patterns is one of the most important factors for the prevention of *Schistosoma japonicum* infection, it is still insufficient for clarifying specific high-risk behaviors and their implications. Parasitological studies and behavioral observations were carried out in a rural village, the Dongting Lake region, China. A time-allocation study conducted by a time-saving spot-check method was implemented to quantify the behavioral risks. Of the 122 participants, 18 (14.8%; 95% confidence interval: 8.5, 21.0) were positive for *S. japonicum*. Among those diagnosed, the median (25－75% quartile) eggs per gram was 8 (8－16). A significant positive correlation with worm intensity was found among people who repair ships on the marshland ($p<0.001$), and this potential risk was consistent with previous suggestions. Although the parasitological techniques and study design require further improvements, our observational methods may be of use to explicitly identify behaviors at the local level that could be relevant to prevention.
Key words: *Schistosoma japonicum;* Schistosomiasis; Water microbiology; Environmental exposure; Behavior; China

1. INTRODUCTION

Schistosomiasis japonica, which is caused by the helminth *Schistosoma japonicum,* is a parasitic zoonosis with more than 40 species acting as definitive hosts [1]. The World Health Organization (WHO) estimates the global number of cases of schistosomiasis due to *Schistosoma* spp. at 200 million, among which 120 million are symptomatic. Moreover, the report states that about 600 million individuals may be at risk worldwide [2]. Since *Oncomelania* snails are still present in numerous areas, and because cattle and buffaloes frequently harbor the infection, schistosomiasis control is a serious challenge even in the 21st century [3].

In mainland China, schistosomiasis japonica remains a major public health problem in eight provinces. Although schistosomiasis gradually decreased after the 1950s and four of 12 provinces succeeded in eliminating the disease, *S. japonicum* was still endemic in 240 counties, with 44 million individuals estimated to be at risk as of 1989 [4]. According to a more recent survey in 2001, 0.8 million humans and 31,500 buffaloes were still infected in China, where the snail habitat area covers 3,436 km^2 [5].

As the disease is mainly transmitted through contact with infected water, it is of practical importance to identify the most risky behaviors and adopt specific preventive measures. In particular, identification of specific water contact at the local level will contribute to reducing the potential risks. Previous studies explored behaviors associated with water contact by means of questionnaires [6,7] and activity diaries [8-10]. But these do not sufficiently clarify behavioral characteristics, and more detailed quantifications, based on direct observation, are required. In this study, we conducted a cross-sectional behavioral study in a rural village, the Dongting Lake region, and analyzed the behavioral risk factors for *S. japonicum* infection that would be relevant for the design of local lifestyle-oriented prevention programs.

2. MATERIALS AND METHODS

2. 1. Study area

Parasitological studies and behavioral observations were carried out in a rural village located in the area called the Dongting Lake region, Hunan Province. Located about

[1]Department of Human Ecology, School of International Health, Graduate School of Medicine, the University of Tokyo, Japan
[2]Research Center for Tropical Infectious Diseases, Nagasaki University Institute of Tropical Medicine, Japan
[3]Hunan Institute of Parasitic Diseases, Hunan, China
[4]National Institute for Environmental Studies, Tsukuba, Japan

120 km from the capital city of Changsha, the village lies northeast of Dongting Lake (Junshan district) where the broad marshland (i.e. a type of wetland, a transition zone between land and water) appears in the dry season. The village is in the vicinity of Dongting Lake (Figure 1). Climatologically, the lake has unique characteristics in that the range of water coverage and water level vary dramatically by season [11]. Human habitation is observed near the dike, where floods were often experienced during the wet season. The village is populated by 1,200 individuals (village leader, personal communication) consisting of three distinct groups. Our study focused on a group of 210 persons. Almost all adults make a living as fishermen, and they are officially allowed to catch shrimp and fresh water fish in the Dongting Lake only between July and October. During other seasons, the villagers find jobs in a distant city and work as migrants or continue fishing under special permission from the government.

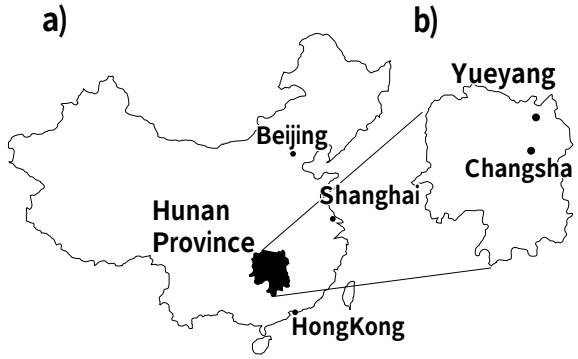

Figure 1. Map of the study area

a) Hunan province is located in the southeast inner area of mainland China.

b) The study area, Junshan district in Yueyang city, is the northern-most part of the province, approximately 120 km from the capital city of Changsha.

2. 2. Methods

2. 2. 1. Study preparation

With the assistance of researchers from the Hunan Institute of Parasitic Diseases, we first identified the climatic and environmental conditions in the study area. According to study members from the local Anti-Schistosomiasis Station, the study area has a higher prevalence of *S. japonicum* infection than other villages, averaging three percent of the population throughout the year. Other areas have succeeded in reducing water contact, mainly as a result of the prevention of floods by dike construction. Since the marshland has been recognized as a particularly high-risk location for potential exposure, we decided to explore behaviors at this location. A previous study also documented that the marshland as well as the lake region were specific areas of risk [6]. In other words, we selected this study area because we could expect characteristic high-risk behaviors to be more obvious here than at other sites.

2. 2. 2. Collection of specimens and ethical considerations

This study was approved by the Ethics Committee at the University of Tokyo and the Hunan Institute of Parasitic Diseases. Before commencement of the study, we informed participants that enrollment was voluntary and gave them the right to withdraw at any time. Each subject was informed as to how the information would be used and assured of the confidentiality of responses. The purpose of the study was explained in Chinese, and written informed consent was obtained from participants.

Subsequently, 137 (65.2%) of the individuals approached agreed to participate in the study and detailed survey. Before conducting the behavioral survey, interviews in Chinese were used to obtain demographic information (i.e. age, sex and occupation). The age of subjects ranged from 6 to 87 years. Single stool specimens were collected from the subjects. The Kato-Katz thick smear technique with 41.7 mg of stools (three slides per participant) was used to measure infestation with *S. japonicum* (eggs per gram of feces; epg). The participants, who clearly remembered having recieved previous treatment, had been given a single oral dose of praziquantel, 40 mg/kg, in 2000 and 2001.

2. 2. 3. Behavioral survey

To explore the behavioral characteristics, time-saving spot-check observations [12] were conducted in October and November 2004. This method records in great detail the time engaged in certain activities and checks the activities of respondents at a scheduled interval in the day. The first author visited the houses of all participants according to a planned time schedule and observed and recorded the activity and location of each participant. The time between 5 am and 7 pm was divided into 14 one-hour intervals and visits were made every other interval on the first day. The intervals not examined on the first day were examined on the second day (Figure 2). Fourteen spot-check records were collected for each individual, or 1708 spot-check observations in total. If a participant was not seen in or around his house when the researcher made a visit, household members or neighbors were asked for his whereabouts. Then, the researcher went to the place to observe his activity there. If the participant had gone to town, no direct observation was made. Continuing this task for two days, almost all activities within the village, including the marshland, had been observed. All observed behaviors were recorded in detail and classified later into 60 categories. In this study, the fol-

lowing 12 behaviors were thought to be particularly high-risk behaviors on the basis of a literature review and discussions in the study location: fishing on the marshland, preparation for fishing on the marshland, repairing ships on the marshland, breeding ducks on the marshland, manual separation of ducks on the marshland, feeding ducks on the marshland, collection of firewood on the marshland, electrical facility fishing, washing, preparation for fishing on dry land, repairing ships on dry land, and working near the fishpond. Electrical facility fishing is defined as an in-river fish sampling method that involves capturing fish using an electric shock technique. As for the last behavior, the fishpond is located outside the dike and has been believed to be safe. The first seven behaviors involve exposure on the marshland, while the latter five are on dry land. Most of the behaviors on the marshland except those related to breeding ducks are conducted when the water level is low and the marshland appears. Therefore most behaviors on the marshland can be observed between October and May, and the villagers had started the behaviors on the marshland about one month before our survey.

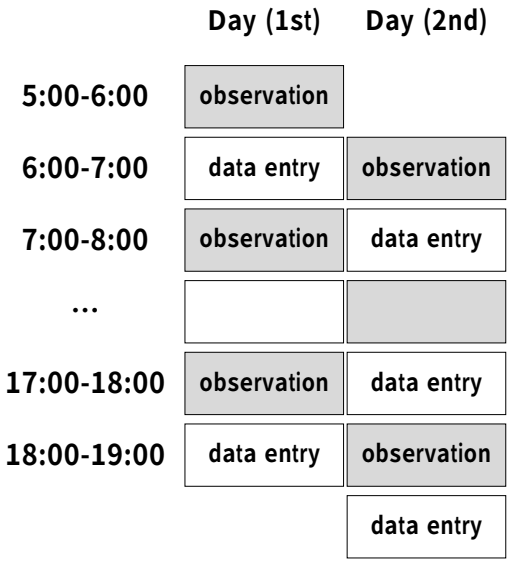

Figure 2. Design of the time-allocation study
Each person was observed for two consecutive days, or 14 hours in total. We repeated observations every other hour. On the second day, we reversed the time for observing and entering the data.

2. 3. Statistical analysis

First, associations between infection and demographic or behavioral variables were examined. Except sex, which is a dichotomous variable, age and time allocations for each of the examined behaviors were measured as continuous variables. Thus, to examine the univariate associations, either the χ^2 test or the non-parametric Mann-Whitney test was used. Second, intensity was used as a dependent variable referring to the geometric mean epg in the population sampled. As the distributions of egg counts were extremely skewed, geometric epg (logarithmic transformation of epg+1) was used instead. To examine univariate association and correlations between the intensity of infection and other explanatory variables, the Mann-Whitney test (for sex) and the Spearman's rank correlation (for other continuous variables) were used, respectively. We used the non-parametric tests because the distributions of both intensity after the logarithmic transformation and the time allocations were skewed to the right. The level of statistical significance was set at α= 0.05. Then, a multiple regression model was used to determine risk factors significantly associated with intensity of *S. japonicum* infection and to eliminate confounding variables. In the multiple regression, we selected the set of variables to be included in the model by the stepwise method. Since there were many potential predictor variables concerning the intensity of *S. japonicum* infection, we selected sex, age, and only variables that were significantly associated or correlated with *S. japonicum* in the univariate analysis.

3. RESULTS

3. 1. Study population and demographic variables

We completed observations for 122 (89.1%) of the 137 individuals who agreed to participate. Nearly one-half were female (n=58; 47.5%). The mean age (and standard deviation; SD) of the individuals investigated was 42.1 (19.5) years. Among these, 18 (14.8%; 95% Confidence Interval (CI): 8.5, 21.0) were positive for *S. japonicum*. Two-thirds (n=12) of those infected were male, although the Mann-Whitney test showed no significant influence of sex on infection (p=0.08). Figure 3 shows the age distributions strati-

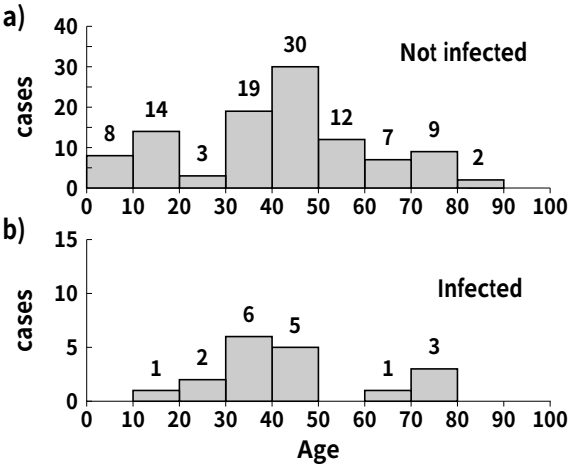

Figure 3. Age distribution by infection with *S. japonicum*
Top: Not infected (n=104; 85.25%). Bottom: Infected (n=18; 14.75%).

fied by infection. Due to the poverty of the community, many young villagers in their twenties had out-migrated to major cities to seek work. As a result, there were only five villagers in their twenties in this village as shown in figure 3. Age was also not associated with infection (p=0.91). Among those diagnosed, the median (25-75% quartile) epg was 8 (8－16). The minimum and maximum epg were 8 and 280, respectively. The geometric epg was neither associated with sex (p=0.49) nor correlated with age (p=0.62).

3. 2. Description of high-risk behaviors

Figure 4 shows distributions of the time-allocation for each behavior by *S. japonicum* infection. Five of a total of seven behaviors on the marshland were performed by participants who were either positive or negative for *S. japonicum*. The participants spent the longest time washing (0.33 hours; 95% CI: 0.22, 0.44), followed by electrical facility fishing (0.25 hours; 0.04, 0.47). On the marshland, fishing (0.12 hours; －0.02, 0.26) and preparation for fishing (0.12 hours; －0.05, 0.29) were the behaviors allocated the long-

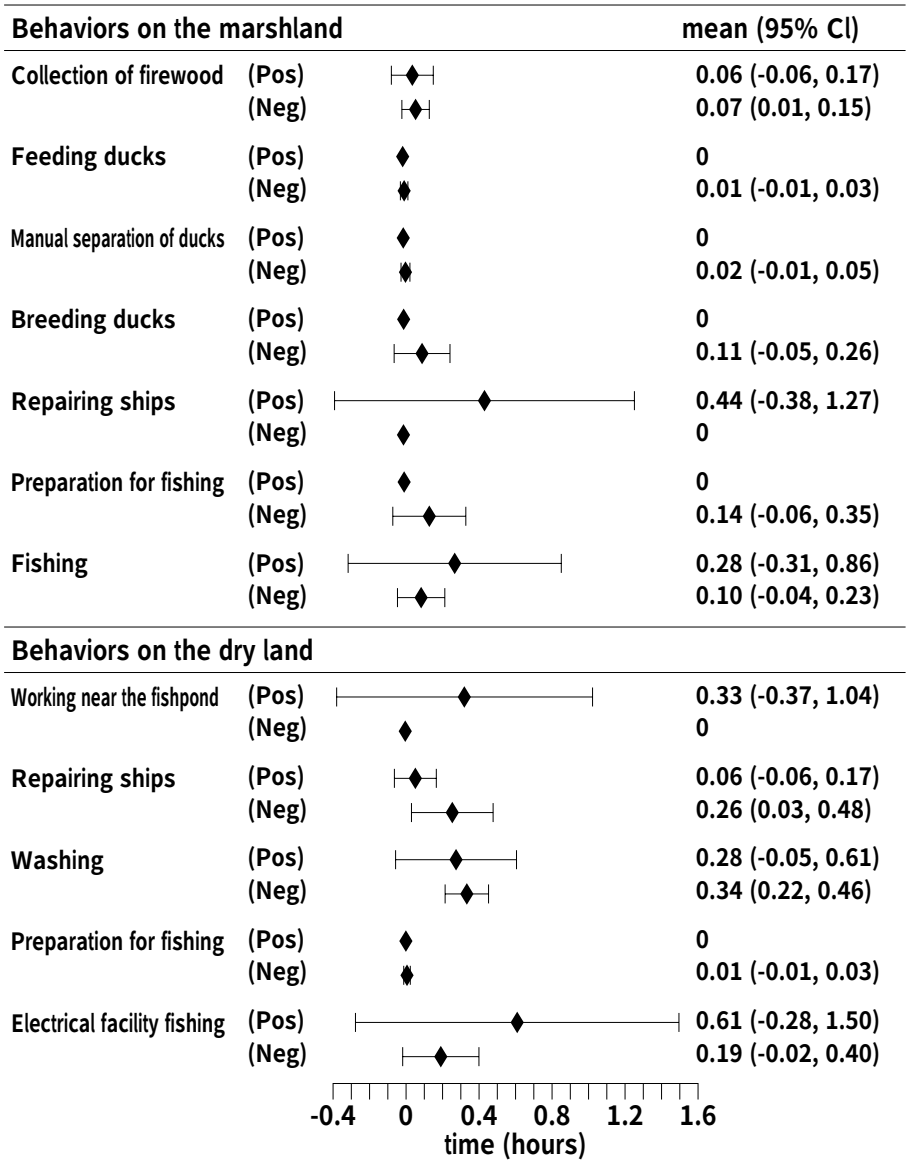

Figure 4. Distribution of time allocated for behaviors on the marshland and dry land
The upper seven behaviors were those observed on the marshland; the lower five were in other areas. Each time-allocation for the observed behaviors was stratified by infection (i.e. 'Pos' and 'Neg' refer to infection and not by means of the single Kato-Katz thick smear method). Diamonds represent the mean time allocated for each behavior. The whisker extends from lower to upper 95% confidence limits.

est times. As a whole, the high-risk behaviors observed were performed for only a few hours per day.

3. 3. Univariate analysis of behavioral factors associated with infection and intensity of *S. japonicum* infection

The Mann-Whitney test revealed that the frequency of infection was significantly higher among those who repaired ships on the marshland ($z=3.40$, $p<0.001$). A similar tendency was seen among those who worked near the fishpond ($z=2.38$, $p=0.02$). Moreover, significant positive correlations with intensity of infection were observed for participants who repaired ships on the marshland (Spearman's $\rho=0.32$, $p<0.001$) and those who worked near the fishpond (Spearman's $\rho=0.20$, $p=0.02$).

3. 4. Multivariate analysis of behavioral factors associated with intensity of *S. japonicum* infection

A multiple regression model (Table 1) shows an overall weak model for predicting *S. japonicum* intensity. This model identified repairing ships on the marshland as the only variable significantly associated with the intensity of *S. japonicum* infection ($p<0.001$). Working near the fishpond and sex were not useful predictors of worm intensity.

Table 1. Multiple regression analysis for *Schistosoma japonicum* worm intensity and behavioral factor-related exposure

Independent variables	Parameter coefficient	S.E.	t	p-value
Intercept constant	0.36	0.09	4.11	<0.001
Sex	0.15	0.09	1.69	0.09
Repairing ships on the marshland	0.75	0.14	5.49	<0.001
Working near the fishpond	0.28	0.16	1.74	0.08

From the reuslts of the univariate analyses, repairing ships on the marshland and working near the fishpond were examined with age and sex by stepwise multiple regression analysis. Then sex, repairing ships on the marshland and working near the fishpond were selected for the final model of the predictor of *S. japonicum* intensity (log [epg+1]). $R^2=0.24$, (F value =12.7, $p<0.001$).

4. DISCUSSION

This study investigated specific behaviors associated with water contact and examined the relationship between the behaviors and infection and intensity of *S. japonicum* in a rural village near Dongting Lake. The study location was unique with regard to the variation in water level by season, which could influence the temporal and spatial spread of the disease [13]. The participants in this study were particularly at risk for potential water contact behaviors on the marshland. According to the local Anti-Schistosomiasis Station, the average prevalence in Junshan district was around three percent (personal communication). The prevalence in this village was higher than the average, but the intensity of *S. japonicum* was low as a whole. The prevalence might be largely influenced by different occupations (which can create heterogeneous patterns), the period of our survey (i.e. seasonal changes in vector ecology and exposure) and mass treatment [14, 15]. Statistical analyses overall did not reveal significant correlations between intensity and the potentially risky behaviors examined. The correlation between working near the fishpond (probably no risk of infection) and *S. japonicum* intensity was significant in the univariate analysis but not significant ($p=0.08$) in the multivariate analysis. Villagers who worked near the fishpond during the survey were fishermen who worked on the marshland on other occasions. Therefore, an apparent correlation was observed between working near the fishpond and *S. japonicum* intensity because working near the fishpond distorted the outcome as confounding factor. In both univariate and multivariate analyses, repairing ships on the marshland was identified as a predictor of *S. japonicum* intensity.

In our direct observation of behaviors, we used a time-saving spot-check method for data collection because it seemed difficult to visit the village, which is located in rural Hunan, to perform repeated observations. Time-allocation observations enabled us to quantify the behavioral data in more detail than questionnaires, 24-hour recall methods or activity diaries. Moreover, a simple questionnaire survey may not be suitable for the identification of behavioral characteristics in detail, because over-reporting of 'correct' behavior (what participants thought they should do) has been claimed in a questionnaire survey related to sanitation and hygiene [16]. With regard to precision and validity, there is a trade-off between exactness of the data and the willingness of subjects to participate in the survey. Since the habitation of the villagers was particularly aggregated, and because their overall participation in the survey was sufficiently high, use of the spot-check method was most suitable for data collection within a limited time period. Consequently, quantifications of the time-allocations for each behavior were reasonable, successful and supported by a valid methodology.

Although the significantly higher risk among participants who repair ships on the marshland may be a straightforward result, it is necessary to discuss the reasons why the other behaviors did not reveal particular relationships with infection and intensity. We attribute this to the following four factors. First, although our study examined infection and intensity by means of the single Kato-Katz thick smear method, it is widely accepted that mild and moderate schistosomiasis japonica can be easily missed when multiple Kato-Katz is not employed [14, 17]. Since it is essential to

measure the relationship between intensity and potential factors, and because *Schistosoma* spp. are macroparasites [18], the low sensitivity of single-field evaluation must be kept in mind [19]. Second, repairing ships on the marshland included the removal of matter clinging to the bottom and side of ships. This activity requires a lot of water. Because of this, repairing ships on the marshland was conducted near water. This is a reason why repairing ships on the marshland was related to both the infection and the intensity. Third, this study differs from previous epidemiologic investigations in that the behaviors were measured in terms of time-allocation (continuous variables); i.e., the statistical correlations between the intensity of infection and exposure doses were examined on the basis of a rough assumption that the dose is proportional to the time-allocation. This anthropological measure enables us to evaluate behaviors quantitatively and qualitatively [12]. But it was extremely difficult to obtain further specific answers in the present setting because of statistical conditions (we examined, not 'associations', but 'correlations' with intensity, which are far less efficient in elucidating detailed relationships) and because most individuals had experienced exposure on the marshland. Finally, the intensity of schistosomiasis is heavily influenced by genetic heterogeneity [20] and acquired immunity [21]. Considering the potential confoundings among the parasitological factors, future epidemiologic studies should incorporate more biological variables in addition to socio-cultural and socio-behavioral factors.

In conclusion, this study attempted to evaluate the details of high-risk behaviors associated with water contact, and it demonstrated a consistent correlation between the intensity of *S. japonicum* infection and exposure on the marshland. In future studies, several key factors as described above should be taken into account to improve understanding of the epidemiologic process of this disease. By elaborating the details of our investigations at both parasitological and anthropological levels, our methodology may be useful to explicit identify behaviors at the local level which could be relevant to specific prevention.

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PREBIOTIC EFFECT OF DAILY FRUCTOOLIGOSACCHARIDE INTAKE ON WEIGHT GAIN AND REDUCTION OF ACUTE DIARRHEA AMONG CHILDREN IN A BANGLADESH URBAN SLUM: A Randomized Double-masked Placebo-controlled Study

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Abstract: Fructooligosaccharide (FOS) is a typical prebiotic agent. A randomized, double-masked, placebo-controlled study was performed to evaluate the prebiotic effect of daily intake of an isotonic solution containing FOS on body weight gain and the reduction of diarrhea in children in an urban slum in Bangladesh over six consecutive months. We enrolled a total of 150 children, aged 25-59 months. Sixty-four children in the FOS group received 50 mL of isotonic solution with 2 g of FOS added, and 69 children in the placebo group were given an identical solution with 1 g of glucose added, once a day. The measurement of body weight was carried out every other day; height and arm circumference were measured once a month; and the children's mothers were interviewed to obtain data about diarrhea, the consistency and constitution of stool, other symptoms, and antibiotic treatment. As a result, the body weight gain during the six-month period was 0.86±0.55 kg in the FOS group and 0.89±0.48 kg in the placebo group, while the increase in height and arm circumference were not significantly different between the two groups. The number of diarrhea episodes during the six-month period was not significantly different. A significant reduction in the duration of diarrhea days and of duration per episode was observed in the FOS group (p =0.039 and p=0.008, respectively). In conclusion, daily intake of FOS was associated neither with the children's growth nor with the number of diarrhea episodes, but a significant reduction in the duration of diarrhea days was observed. Further studies are needed to confirm the effects of FOS by changing the doses and eliminating the influence of antibiotics.

Keywords: prebiotic effect, fructooligosaccharide (FOS), weight gain, diarrhea, RCT

INTRODUCTION

Diarrhea is a severe health problem that leads to mortality among children in developing countries. Clinical studies have clarified that some nondigestible carbohydrates with a molecular weight of more than 20,000 can alleviate diarrhea [1-8]. Fructooligosaccharide (FOS) is a mixture of 1-kestose (GF2), 28%; nystose (GF3), 60%; and β-fructofranosyl nystose (GF4), 12%; [9, 10]. It is completely safe [7, 9], has a sweet taste, and is easy to dissolve due to its small molecular weight (approximately 680). Prebiotics have already been identified as nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth or activity of one or more bacteria in the colon [11, 12].

FOS is a typical nondigestible oligosaccharide and prebiotic agent. It is not hydrolyzed by any intestinal enzymes, is well fermented in the large intestine in human studies [13, 14], and improves the intestinal microflora in such a way that it becomes difficult for pathogenic microbes to proliferate in the human gastrointestinal tract [15-20]. The fermentation brings probiotic effects *in vitro* [21, 22], *in vivo* [21, 23], and also in humans [21, 24]. Using [U-^{14}C] FOS Oku, et al. have already shown that more than 99% of FOS is metabolized through fermentation by intestinal microbes [10, 25, 26]. The final products of fermentation are gases and short chain fatty acids such as acetate, propionate, and *n*-butyrate [10, 25, 27, 28]. Through the process of fermentation, harmful products such as ammonia, phenol, skatole, and indole are reduced due to the reduction of the en-

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zyme activity needed to synthesize these products [29, 30].

Furthermore, studies using animals and humans to determine the interaction of FOS supplementation with gastrointestinal function [31, 32], growth [29], immune response [33, 34], diarrhea in pigs [35], and traveler's diarrhea [36] have been assessed. However, the effect of FOS intake on the reduction of diarrhea and growth in children in the community has not been clarified [37]. Our hypothesis is that the daily intake of FOS-supplemented commercially developed isotonic solution improves the intestinal microflora and reduces diarrhea, thereby inducing an improvement in body weight gain in children. To assess the prebiotic effect on body weight gain and on the reduction of diarrhea, we performed a randomized, double-masked, placebo-controlled study for six consecutive months with children in an urban slum community of Bangladesh.

SUBJECTS AND METHODS

Study Population

The trial took place in Mirpur, an urban slum community with 800 households in the city of Dhaka, Bangladesh between December 2004 and June 2005. Mirpur is densely populated and has poor sanitary and hygienic conditions. Diarrhea is known as a serious health problem among children in the slums of Dhaka. The International Center for Diarrhea Diseases Research (ICDDR, B) has established a demographic surveillance system in this area, and a sampling frame was available. Children aged 25-59 months and their mothers were recruited randomly based on the demographic database prepared by ICDDR, B. Children who were being breast-fed, those who suffered from chronic diarrhea or malnutrition (weight-for-height z-score <-2), and/or those who were receiving antibiotic therapy were excluded.

A total of 158 children were enrolled, but 8 out of 158 did not participate. Therefore, 150 children were randomly assigned to each of two groups, the prebiotic (FOS) or the placebo (glucose) group.

The trial was approved by the Ethical Committee of Siebold University of Nagasaki Prefecture, and by the Ethical Review Committee of ICDDR, B. Informed consent was obtained from all parents. The children were provided medical care in the ICDDR, B-run clinic for any serious illnesses during the study period. Oral rehydration therapy was performed for most of the diarrhea cases observed.

Study Outcome

The primary study outcome was body weight gain and a reduction in the number of diarrhea episodes. The secondary outcome was a decrease in the cumulative duration of diarrhea days, the duration of diarrhea days per diarrhea episode, and the number of defecations per day during a diarrhea episode. According to the World Health Organization [38], diarrhea is defined as the passage of three or more loose or watery stools in a 24-h period. An episode of diarrhea was defined as a period beginning with a day when the subject experiences more than three loose stools and ending with the last diarrhea day followed by at least two consecutive days without diarrhea. Severe diarrhea was defined as either persistent or invasive diarrhea. Persistent diarrhea was defined as having a duration of more than 14 days, while invasive diarrhea was defined as diarrhea with macroscopic blood.

Study Protocol

The intervention involved the daily intake for six consecutive months of commercially produced isotonic solution (Pocari Sweat, Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) with the addition of fructooligosaccharide (FOS, Meiji Seika Kaisha Ltd., Tokyo, Japan), the chemical structure of which is 1^{F}-(β-fructofranosyl)$_{n-1}$-sucrose, where n varies from 2 to 4 (e.g., 2, 1-kestose (GF2), 28%; 3, nystose (GF3), 60%; and 4, 1^{F}-β-fructofranosyl nystose (GF4), 12%). The placebo group received an identical bottle of placebo solution with glucose (Nihon Shokuhin Kako CO., Ltd., Tokyo, Japan). FOS and glucose were of an analytical grade. The commercially produced isotonic solution masked the slight differences in both sweetness and taste between the two solutions. In order to reduce the osmotic pressure of the solution, 1.85 g of "Pocari Sweat" powder was dissolved in 50 mL of water, and 2 g of FOS or 1 g of glucose was added to achieve equal energy. The total osmotic pressure was 0.234 Osmol/kg for the FOS solution and 0.278 Osmol/kg for the glucose solution, measured by the cryoscopy freezing-point method using the Osmometer OM802 (Asahi Life Science Co., Ltd., Tokyo, Japan). The main nutrients and the concentration of electrolytes in these solutions are shown in Table 1. Forty mL of each test solution was freshly prepared every morning, and then 50 mL was transferred to a bottle for each child.

Prior to the start of the trial, randomization was carried out using the master randomization code, and two sets of code envelopes were made by an independent statistician, who was not involved in the study in any way. No collaborator or field research assistant knew the group to which any of the children belonged, or the content of any particular solution bottle.

Four trained field research assistants and four health attendants were recruited from the study area. A field research assistant and a health attendant formed a team for house visits and confirmed the screening criteria. Four

Table 1. Nutrients and concentration of electrolytes in FOS and placebo solutions

	FOS solution	Placebo solution
Nutrients (per 100 mL)		
Energy (kcal)	16.0	16.0
Carbohydrate (g)	3.4	3.4
Protein (g)	0.0	0.0
Fat (g)	0.0	0.0
Added FOS (g)	4.0	0.0
Added glucose (g)	0.0	2.0
Electrolytes (mmol/L)		
Na^+	21.0	21.0
K^+	5.0	5.0
Ca^{2+}	0.5	0.5
Mg^{2+}	0.25	0.25
Cl^-	16.5	16.5
$Citrate^{3-}$	3.3	3.3
$Lactate^-$	1.0	1.0

FOS, fructooligosaccharide.
Data were obtained from Otsuka Pharmaceutical Co., Ltd.

teams distributed the test solution daily and directly administered it to each child. They interviewed the mother of each child every day, and measured each child's body weight on alternative days. The interview questions were the number of defecations, consistency and constitution of stool (hard, loose, or watery; visible blood and/or mucus in the feces), abdominal or other symptoms, and treatment and administration of antibiotics. The research assistants conducted their activities according to a manual using checklists. If the child was not at home, the mother was asked to keep one bottle and to give it to the child within a day. Body weight was measured using a digital scale with 0.1-kg precision (UNI-scale). Height and arm circumference were measured using a wooden perpendicular scale and a TALC tape measure, respectively in the field clinical office once a month. Measurements were carried out at least two times.

Data Management and Statistical Analysis

The study releaved a 0.06 kg mean difference with a 0.12 kg standard deviation in body weight gain, based on the assumptions from previous studies in Bangladesh [39, 40], using a two-sided alpha of 0.05 and a power of 80%. The requirement of more than 63 children per group was established, and assuming a 16.7% (20/120) drop-out rate, the calculated number of children required for the study was 75 in each group.

Data were collected by the field research assistants according to forms that were reviewed by the supervising staff in ICDDR, B. Data were entered and checked logically and in terms of range. Thereafter, cleaning and analysis were performed using SPSS ver.11 for Windows, Japan (SPSS Inc., Japan). For comparison of the categorical data, chi-square or Fisher's exact test was used. Continuous data were compared with Student's *t*'test for normally distributed data and the Welch or Mann-Whitney *U* test for non-parametric data. Pearson's correlation was used to evaluate the relation of the number of diarrhea episodes or cumulative days of diarrhea with the body weight gain. The data were expressed as means and standard deviations with the significance level considered to be less than 0.05.

RESULTS

Characteristics of Children at Baseline

The flow chart of randomization procedure is shown in Figure 1. The characteristics of the participants initially allocated to each group and those who completed the study are shown in Table 2. The characteristics were not significantly different for the initial groups. Age in the FOS group (n=75) was 46.4±9.8 months, and that in the placebo group (n=75) was 46.5±9.2 months. The number of children who completed the study was 64 in the FOS group and 69 in the placebo group. The percentage of males was significantly higher in the FOS group than in the placebo group (p=0.037).

Dose Levels of FOS Administered

According to the field research assistants, all the children liked to drink the solutions, and none refused the administration. The average dose level of the administration of FOS was 0.147±0.235 g per kg of body weight at the start of the study period and 0.138±0.222 g per kg of body weight at the end of the study.

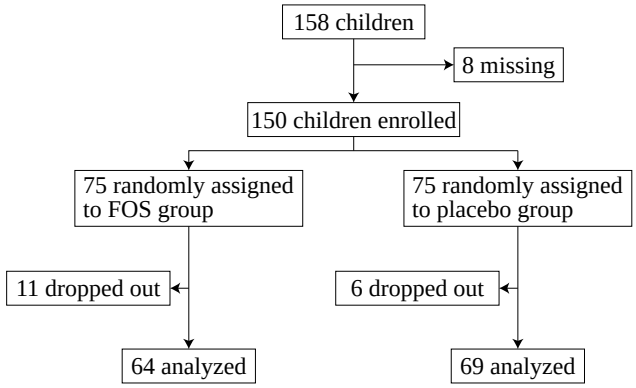

Figure 1. Flow chart of randomization procedure and attrition of study participants

Table 2. Characteristics of children at baseline: enrolled and analyzed

	Initially allocated		Completed study	
	FOS (n=75)	Placebo (n=75)	FOS (n=64)	Placebo (n=69)
Age (mo)	46.5 (9.2)	46.4 (9.8)	46.5 (9.1)	46.3 (9.4)
Males (%)	52.0	38.6	53.1[a]	34.8
Body weight (kg)	12.8 (2.0)	12.6 (2.0)	12.9 (2.0)	12.6 (2.1)
Height (cm)	94.9 (8.6)	93.8 (7.8)	95.0 (8.3)	93.8 (8.1)
MUAC (cm)	15.0 (1.0)	15.1 (1.1)	15.0 (1.1)	15.1 (1.2)

FOS, fructooligosaccharide; MUAC, arm circumference. Values were expressed as mean and (standard deviation: S.D.). The number of enrolled children was 150; a group of 75 children were allocated to the FOS-group, and the other 75 were to the placebo group. Sixty-four children of the FOS-group and 69 children of the placebo group completed the study and their data were used for analysis. a: significantly different from the placebo group, at p=0.037.

Growth and the Number of Children Experiencing Diarrhea

Data on body weight gain after six months' daily intake of the two kinds of test solutions are shown in Table 3. The average body weight gain in the FOS group was 0.86±0.55 kg, while that in the placebo group was 0.89±0.48 kg. There were no significant differences in the increase in body weight, height and arm circumference between either the FOS and placebo groups or the males and females in each group.

The number of children who experienced one or more episodes of diarrhea was 39 out of 64 children in the FOS group and 44 out of 69 children in the placebo group. The difference between the groups was not significant. The number of children experiencing persistent diarrhea was only one in the FOS group and two in the placebo group.

The Number and Duration of Diarrhea Episodes

Table 4 shows the average number of diarrhea episodes, the cumulative diarrhea days over the six-month period, the duration of diarrhea per episode, and the number of defecations per day of diarrhea. The average number of diarrhea episodes was 1.3±1.6 in the FOS group and 2.0±2.8 in the placebo group. There were apparently fewer episodes of diarrhea in the FOS group than in the placebo group, but the difference was not significant.

The cumulative number of diarrhea days in the FOS group was 3.3±4.9 days, significantly fewer than that in

Table 3. Growth during the six-month period and the number of children who experienced diarrhea

	FOS (n=64)	Placebo (n=69)
Body weight gain (kg)	0.86 (0.55)	0.89 (0.48)
Height gain (cm)	2.76 (0.71)	2.73 (0.68)
MUAC gain (cm)	0.24 (0.39)	0.27 (0.41)
Children reporting diarrhea (number)[*1]	39	44
Children reporting persistent diarrhea (number)[*2]	1	2

FOS, fructooligosaccharide; MUAC, arm circumference. Values were expressed as mean and (S.D.). [*1]: the number of children who experienced one or more episodes of diarrhea; [*2]: the number of children who experienced persistent diarrhea. No difference was observed between the two groups.

Table 4. The comparison of diarrhea episodes between FOS and placebo groups

	FOS (n=63)	Placebo (n=67)	p-values
Number of diarrhea episodes (number)	1.3 (1.6)	2.0 (2.8)	0.098
Cumulative diarrhea days	3.3 (4.9)	6.3 (10.8)[a]	0.039
Duration of diarrhea days per episode[*1]	2.5 (1.8)	3.2 (2.4)[b]	0.008
Number of defecations per day of diarrhea[*1]	2.5 (1.7)	2.2 (1.4)	0.096
Visible blood in feces (number)	1	1	1.000
Mucus in feces (number)	9	14	0.365

Values were expressed as mean and (S.D.). The total numbers (64 in FOS group, and 69 in placebo group) excluding persistent diarrhea of FOS and placebo groups were 63 and 67, respectively. [*1]: n=84 in FOS group, and n=147 in FOS group, respectively. a, b: significantly different from the placebo group, respectively.

Table 5. Other diseases, symptoms and antibiotic treatment during the six-month period

	FOS (n=50)	Placebo (n=51)
Measles	1	2
Cough	44	44
Angular stomatitis	49	51
Ear discharge	50	51
Vomiting	15	18
Antibiotics treatment	50	51

No significant difference was detected between the two groups.

the placebo group (6.3±10.8 days), at p=0.039. The duration per diarrhea episode in the FOS group (2.5±1.8) was also significantly shorter than that in the placebo group (3.2 ±2.4), at p=0.008. However, the number of defecations per days of diarrhea was not significantly different between the FOS group (2.5±1.7) and the placebo group (2.1±1.4).

The number of children with macroscopic blood in feces was one in each group. Mucus in feces occurred in nine children in the FOS group and 14 in the placebo group. The difference was not statistically significant.

Other Symptoms and Antibiotic Treatment

The data on other symptoms were collected for 50 out of 64 children in the FOS group and for 51 out of 69 children in the placebo group, as shown in Table 5. Cough was observed in 44 out of 50 children in the FOS group and 44 out of 51 children in the placebo group. The difference was not significant. Most of the children experienced angular stomatitis and ear discharge. Chloramphenicol antibiotic treatment was administered to almost all of the children in the two groups.

Association of Diarrhea with Body Weight Gain

The number of episodes and duration of diarrhea had no significant correlation with body weight gain in either the FOS (r=-0.092, r=-0.64) or placebo group (r=-0.088, r= -0.44).

DISCUSSION

The prebiotic effect of daily FOS intake on growth and on the episodes and duration of diarrhea was studied in free-living children in an urban slum in Bangladesh. The difference in growth between the FOS and placebo groups was not significant, nor did the number of diarrhea episodes differ between the two groups. Many factors other than the prebiotic effect might directly and/or indirectly affect growth and diarrhea. It has been reported that it is difficult to detect the benefit of the prebiotic effect in healthy populations [36, 37].

Three factors may explain this. First, the dose of FOS, on average 0.147 g per kg of body weight, might be too small to reduce the number of diarrhea episodes. We selected the dose on the basis of the following two points: 1) dose transitory diarrhea can be completely avoided; and 2) can promote the intestinal microflora. In adults, approximately 15 g of daily single intake (or 0.3 g per kg of body weight) may induce hyperosmotic transitory diarrhea [26, 41, 42], and 1-2 g of daily single intake improved intestinal microbes [15]. Considering that 0.5-2.5 g of nondigestible oligosaccharides is supplemented to 100 g dry weight of some brands of artificial formula milk in Japan (0.05-0.3 g per 100 mL of formula solution), it may be possible to test higher doses of FOS to examine the prebiotic effects in the future. Further studies are needed to identify the optimal dose level of FOS for children.

Secondly, most children were reported to have symptoms of cough, angular stomatitis, and/or ear discharge and to be under treatment with chloramphenicol antibiotics, which interfere with the proliferation of not only harmful but also beneficial microbes [25, 27, 43, 44]. Chloramphenicol is a broad spectrum antibiotic, and it has been shown to reduce intestinal microbes in rats [25]. The prebiotic effect might be disturbed by the use of antibiotics.

Thirdly, this study was conducted from the cool and dry winter season to the hot and rainy season of Bangladesh. Since many of the children had been treated with antibiotics for respiratory infectious diseases in winter, the result may have differed if the study had started in the rainy season.

On the other hand, the average number of days of diarrhea during the six-month period and the number of days of diarrhea per episode were shorter in the FOS group than in the placebo group. Intake of FOS may reduce the duration of diarrhea in the children. But the shorter duration of diarrhea did not relate with weight gain, presumably because of the effects of antibiotics. Further studies are required to determine the prebiotic effect of FOS under conditions in which antibiotics effects have been eliminated. The optimal dose level and affordable administration methods of prebiotics for mitigating the disease burden of diarrhea should be also studied.

In conclusion, daily intake of FOS was associated neither with the children's growth nor with the number of diarrhea episodes, but a significant reduction in the duration of diarrhea days was observed. Further studies are needed to confirm the effects of FOS by changing the doses and eliminating the influence of antibiotics.

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