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Establishment of Bio-medical Laboratories in the Kenya Research Station and a Diarrheal Disease Research Project in Kenya

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PREPARATION FOR LABORATORY WORK

Establishment of Laboratories

The Nagasaki University Institute of Tropical Medicine (NUITM) Kenya Research Station was established in 2005 as a five-year research project supported by the Ministry of Education, Culture, Sports, Science and Technology of Japan to promote the development of research on emerging and re-emerging infectious diseases and tropical infectious diseases.

Construction of bio-medical laboratories is an important part of our project in that it will enable us to provide scientific evidence of infectious diseases. Bio-medical laboratories were established in the Centre for Microbiology Research (CMR) at the Kenya Medical Research Institute (KEMRI) by the end of 2006 under an agreement between KEMRI and Nagasaki University. The laboratory aspect is essential, and it will be a core component for our project. Laboratory work will not only provide essential data for diagnosis and analysis of pathogens but will also complement epidemiological data and information from the field.

It is hoped that the laboratory will meet international standards, fulfill the needs of public health, and respond to health threats such as emerging and re-emerging infectious diseases. In order to ensure biological safety in handling pathogenic microorganisms, all biomedical research laboratories will be fully prepared and certified.

Since the NUITM-KEMRI office was set up to implement the concept of biosafety in the laboratories, some parts of the CMR laboratories have been renovated and newly equipped. These are biosafety level 2 (BSL2) laboratories, biochemical and molecular laboratories for research in the fields of virology, bacteriology, protozoology and parasitology. In addition, the establishment of a BSL3 laboratory in CMR was planned to be established until March 2007.

Establishment of the BSL3 Laboratory

The BSL3 laboratory is one of the most important components of our project, particularly when BSL3-level pathogens such as West Nile fever, yellow fever and multiple drug-resistant *Mycobacterium tuberculosis* are the subject of research and when etiologically unknown specimens are investigated in the course of our research project.

The export of the BSL3 laboratory from Japan and its construction in Kenya posed various difficulties. For example, it took a long time to obtain an export permit from Japanese authorities because of the need for absolute assurance that the laboratory will not be used for biological terrorism. Since this was the first time for a Japanese university to grapple with this problem, we outline our experiences here as a history.

The scheme for the exportation and construction of a BSL3 laboratory as part of our project was approved by the committee of NUITM in June 2006. Immediately after approval, the construction of the BSL3 laboratory was advertised and international tenders invited. A construction company and trading company were selected, and Nagasaki University entered into contractual agreements with these companies. The concrete plans for construction of the BSL3 laboratory in CMR were arranged on the basis of a spot investigation carried out in September 2006. Before the scheduled transportation to Kenya in January 2007, the BSL3 laboratory system was constructed and assembled provisionally in the construction company yard in Osaka to test performance. We applied to the Ministry of Economy, Trade and Industry for an export permit only to learn that ours was the first case of a university asking to export government-controlled goods. Once the export permit was

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obtained, we packed and shipped the whole system to Kenya and, at the same time, filed a request with the Kenyan Ministry of Finance for tax and duty exemptions. After a three-week long custom clearance process, the system was transported to CMR, KEMRI and assembled within two weeks. The construction of the BSL3 laboratory finally reached completion in the end of March 2007, meaning that it took a whole year from inception to completion to realize the goal of our project to establish a BSL3 laboratory. The BSL3 laboratory is now being prepared for operation and use. Improvements will be made continually to the laboratory operation.

DIARRHEAL DISEASE RESEARCH IN KENYA

Introduction

Diarrheal diseases or enteric infectious diseases remain a major public health concern in developing countries and cause a high rate of mortality, especially among young children. Each year there are an estimated 4 billion cases of diarrheal diseases. Presently, the risk of contracting diarrheal diseases in sub-Saharan Africa is five-fold that in developed countries [1]. According to World Health Organization statistics for the year 2001, diarrheal diseases were the second leading cause of disability-adjusted life year (DALY) lost and were the third leading cause of death worldwide, accounting for 2.2 million deaths or 4% of all deaths [2].

Although diarrheal disease is still the most serious threat, especially in the tropics, HIV/AIDS is currently rampant, requiring focused and specific attention. The magnitude of diarrheal diseases should be reconsidered in the HIV /AIDS pandemic areas, resources reallocated, and public attention drawn to the importance of preventive efforts.

Background

In Kenya, the number of diarrheal cases may be decreasing year by year, but details concerning the etiological agents of diarrhea are not fully known at present in either urban or remote areas [3].

In particular, enteropathogenic *Escherichia coli* is one of the most important etiologic agents of childhood diarrhea and represents a major public health problem in developing countries [4]. The diagnosis of diarrheagenic *E. coli*, however, depends upon the identification of virulent factors, such as toxins and invasive factors. Serotyping of O antigen is not sufficient to identify a virulent strain, because it does not necessarily correlate with the possession of virulent factors [5]. The actual status of enteropathogenic *Escherichia coli* infection is not well understood.

In Kenya, enterohemorrhagic *Escherichia coli* (EHEC) is rarely isolated as a causative agent of diarrhea as in other developing countries [6]. Most EHEC outbreaks occurring in developed countries and outbreaks of EHEC infection in tropical areas have not been reported so far. The etiological investigation of diarrheagenic agents, focusing on EHEC, may not have been thoroughly attempted because of the complexity of isolation techniques and the lack of facilities. Immunological analyses may be needed to answer the question as to why EHEC infections are fewer in developing than in developed countries.

Gastroenteritis due to *Campylobacter jejunii* is also of importance to public health [7]. However, the details of its infection in Kenya have not been fully clarified because it had been reported to be a diarrheagenic enteropathogen [8]. The pathophysiology of Campylobacter infection is an acute inflammatory bowel disease like shigellosis, but the production of enterotoxin from the organism was previously reported [9, 10]. It has also been suggested that it has the potential to induce systemic infection and result in a high mortality, especially in compromised hosts such as HIV/ AIDS patients. Furthermore, the drug resistance of *Campylobacter jejunii* to highly effective new quinolones may raise serious problems and therefore should be carefully monitored.

Etiological investigations into diarrheal diseases focusing on *Aeromonas* sp., *Plesiomonas* sp. and *Vibrio* sp., such as *Vibrio parahemolyticus, Vibrio mimicus, and Vibrio vulnificus,* have also not been thoroughly conducted in Kenya.

Norovirus is also a diarrheagenic viral agent that has drawn much attention recently. Gastroenteritis due to norovirus is self-limiting, with high infectivity, accounting for more than 50% of diarrheal disease in developed countries.

A new diarrheagenic agent called *Providencia alcalifaciens,* a member of the *Enterobacteriaceae* family, has also emerged [11], but the status of these infections are yet to be elucidated either in urban areas or areas highly infected with HIV/AIDS.

Cholera is currently one of the most important re-emerging infectious diseases. Sub Saharan African countries including Kenya have reported more cases than any other part of the world. *Vibrio cholerae* is indigenous to the estuarine environment, even though it is a human pathogen. What role does the natural water environment play in the survival or acquisition of dominant character in a new strain?

In 1991, serotype O139 emerged as a new epidemic strain, although until then serotype O1 was the only known serotype of cholera. The emergence of *Vibrio cholerae* O 139 strain raised interest in *Vibrio cholerae* non-O1 in the environment. In fact, when environmental water surveillance was performed in Kenya in 1984 immediately after the subsiding of a cholera outbreak, the isolates from envi-

ronmental water were not only O1 *Vibrio cholerae* but also non-O1 *Vibrio cholerae* [13]. The water environment and the non-O1 *Vibrio cholerae* residing there may be important key factors backstage where vibrios can transform into a new strain and acquire virulent characteristics.

Research Objectives

1) Epidemiology of diarrheal diseases

Extensive etiological investigations regarding diarrheagenic agents will be performed in the remote areas where HIV/AIDS prevalence is high and where a demographic surveillance system (DSS) has been established. The precise incidence of diarrheal disease will be identified, and the degree of vulnerability and seriousness of diarrheal diseases such as shigellosis, enteroinvasive *E. coli* in the HIV-positive group will be compared to those in the HIV-negative group. The diarrheagenic agents unidentified to date will be clarified in this study along with viral and newly recognized diarrheagenic agents. The details of enteropathogenic *E. coli* infection in Kenya will be elucidated using multiplex PCR [12].

2) Research on transmission routes of infectious diseases

Hospital-based etiological examination of diarrheal diseases will be performed in urban settings like Nairobi. When the outbreak of an enteric infectious disease including food poisoning is detected, a case control study will be conducted to identify and verify the transmission route of the infectious disease or food poisoning.

3) Research on enterohemorrhagic *Escherichia coli* infection

Enterohemorrhagic *Escherichia coli* (EHEC), which causes sporadic cases and occasionally even severe outbreaks in developed countries, is rarely isolated as a causative agent of diarrheal disease in developing countries (6). In this study, we will not only conduct an etiological investigation regarding diarrheal cases but also determine the carrier rate of EHEC among non-diarrheal cases and among domestic animals such as cattle, pigs and sheep. In addition, serological analyses of the serum IgG level of anti-VT1, anti-VT2 and anti-O157 LPS antibodies among Kenyan and Japanese pediatric cases will be undertaken to clarify the sero-dynamics of EHEC infection.

4) Research on epidemiological tools for prediction of cholera outbreaks

People in cholera-infected areas are under the constant threat of cholera outbreak and continue to die from the disease even though the treatment of cholera has been established. If a cholera outbreak can be predicted, the authorities can deliver a warning to people in the infected areas to take countermeasures. If the danger of an outbreak can be detected by monitoring in the environment, the risk of a cholera outbreak can be reduced. The genotypic differences of virulent factors, focusing on vibrio phages particularly among *Vibrio cholerae* non-O1 isolated in the environment, will be analyzed and monitored so that molecular tools associated with the acquisition of new character can be identified.

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