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EXPERIENCE AND REVIEW OF SARS CONTROL IN VIETNAM AND CHINA

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Accepted 7, June, 2004

Abstract: Severe Acute Respiratory Syndrome (SARS) has rapidly spread and caused epidemics in many countries. During these epidemics, the Japanese government contributed to SARS control by dispatching medical teams. In the present article, the author reviews and discusses the process of control of the SARS outbreak based on experiences in activities to support SARS control in Vietnam and China.

Vietnam succeeded in the effective control of SARS for the first time in the world. This was accomplished by complete isolation of patients and implementation of nosocomial infection control from an early stage of epidemic, etc. In China, due to inadequate response in the early stage, nosocomial infection occurred frequently and the disease quickly spread. However, later, effective actions were taken under the strong direction of the government, and the disease was finally put under control. The Japan Medical Team for Disaster Relief dispatched by the government of Japan to Hanoi, Vietnam and Beijing, China offered cooperative activities for the prevention of nosocomial infection and respiratory management. In addition, the Medical Aid Team sent to Guangdong Province in China provided guidance to the local Japanese residents to prevent the infection of SARS and to alleviate anxiety about the disease.

In the control of SARS, it is essential to take adequate actions from an early stage in the development of the disease. For this purpose, rather than starting measures for the control of nosocomial infection after the eruption of the disease, it is important to train medical staff on a routine basis, to establish a nosocomial infection control system, and to consolidate basic preventive practices.
Key words: SARS, Vietnam, China, nosocomial infection control, Japanese residents

INTRODUCTION

Originating from Guangdong Province in China, Severe Acute Respiratory Syndrome (SARS) quickly spread in many countries and regions, including Hongkong, Vietnam, various provinces in China, Taiwan, and the whole world was struck with horror [1-3]. In many hospitals where SARS patients were admitted, nosocomial infection frequently occurred, accelerating the epidemics [4-6]. In Vietnam, adequate measures to prevent nosocomial infection were taken from an early stage and SARS was successfully controlled earlier than in other countries. At that time, infection was still spreading in China, and confusion and dismay still prevailed. However, despite a serious delay in taking proper actions in the early stage, China enforced intensive measures under the strong leadership of the government, and the disease was finally contained. During the period of epidemics, there was a feeling of extreme anxiety and uneasiness among Japanese residents in these areas.

To assist in the control of SARS epidemics and health management among Japanese residents, the government of Japan dispatched three medical teams, all of which the author joined. The cooperation activities were deployed in Hanoi City, Guangdong Province, and Beijing City. In the present article, the author attempts to review the course of the SARS epidemics in Vietnam and China and the procedures taken for disease control in association with Japanese cooperation based on the experience of participation in the above teams.

REVIEW OF SARS OUTBREAKS AND CONTROL

1. Vietnam

1-1 General aspects of the SARS outbreak

The first case of SARS in Vietnam occurred on February 26, 2003. The patient was a 48-year-old Chinese-American businessman who had been infected with the disease in Hongkong. The patient was admitted to F Hospital in Hanoi, a facility with 75 beds primarily offering medical service for foreigners. In this hospital, nosocomial infection began to develop in rapid succession from early March. Ultimately, 63 persons were infected with the disease in-

Bureau of International Medical Cooperation, International Medical Center of Japan

cluding 39 hospital staff (65%), hospital visitors and patients' family members [7]. The Ministry of Health took actions to stop the admission of all new cases to F Hospital. On March 11, Bach Mai Hospital (BMH) was designated as the sole hospital to provide medical care for SARS cases in Vietnam. Subsequently, all newly infected patients (including those who had a history of contact with F Hospital in some way or other) and SARS patients from F Hospital were moved to BMH.

In BMH, no case of nosocomial infection occurred and the SARS epidemic in Vietnam gradually subsided. On April 28, 2003, the Ministry of Health and World Health Organization (WHO) declared SARS under control in Vietnam [8]. All patients admitted to BMH recovered to normal condition (no death case), and the last of the two SARS cases was discharged from the hospital on May 2. In Vietnam, a total of 63 patients contracted SARS since the detection of the first case, and five of the patients died of the disease. All of these cases had been derived from a single patient (Figure 1).

1-2 Actions taken for the control of SARS outbreak

In BMH, 35 patients with SARS (including suspected cases) were treated from the start of acceptance of SARS cases on March 11, 2003, to the end of April. During this period, there was no case of nosocomial infection. Observing that nosocomial infection occurred frequently in F Hospital where the SARS cases were admitted in the early stage of the epidemic, the Nosocomial Infection Control Committee of BMH quickly recognized that SARS was "a new type of pneumonia, which showed high infectious potency with strong pathogenicity and which must be observed under special vigilance and care". After BMH began to accept the SARS cases, these cases were isolated in a special ward for infectious diseases, and strict warnings were given to hospital staff. An nosocomial infection control program was set up, aiming at the efficient control of SARS, and was promptly put into motion (Photo. 1).

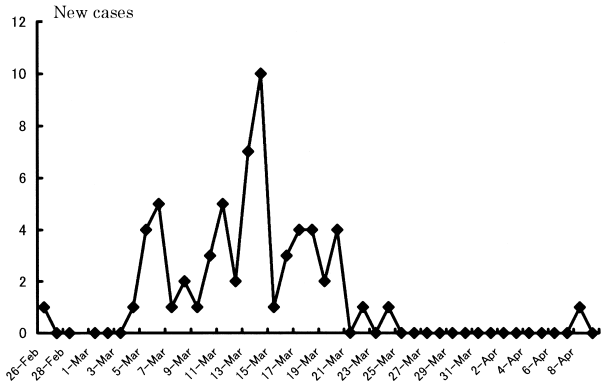

Figure 1. Occurrence of SARS cases in Hanoi, Vietnam.
The first patient was admitted to F hospital on February 26, 2003, where nosocomial infection occurred (65 people including39medical staff were infected). Bach Mai Hospital (BMH) began to accept SARS patients on March 11. A global health alert was issued by WHO on April 12 and 14. No nosocomial infection occurred in BMH, and SARS was declared under control on April 28. The Japan Disaster Relief Team was dispatched from March 16 to April1.

In addition to the standard precautions carried out for the purpose of nosocomial infection control, preventive measures were taken by giving full consideration to the elimination of risks of droplet infection and direct contagion. Those persons who were near or in contact with the patients were instructed to take special attention to the risk of droplet infection, and a strict warning was issued to all medical staff with the possibility of contact with the patients. They were instructed to put on masks, protective gowns, gloves, face shields, and goggles. Because a negative pressure room was not available, the windows of patient rooms were kept open for ventilation.

1-3 Cooperation activities from Japan

1-3-1 Before the SARS epidemic.

BMH is a general hospital with 1,400 beds located in Hanoi, the capital of Vietnam. Since its establishment in 1911, BMH has contributed greatly to medical care of local inhabitants as a core hospital in the northern region of Vietnam. In BMH, a technical cooperation project (the Bach Mai Hospital Project for Functional Enhancement) has been implemented there with aid from the government of Japan for a period of five years since January 2000 (i.e. three years and three months before the SARS outbreak in Vietnam). It aimed to improve the quality of medical service at BMH and to disseminate the benefits of medical care in the northern region of Vietnam [9]. In this project, guidance on

Photo. 1. A medical doctor in complete protective attire treating a serious SARS case (Courtesy of Bach Mai Hospital).

nosocomial infection control was incorporated as one of the technical cooperation programs under the recognition that adequate implementation of nosocomial infection control is essential for the improvement of the quality of medical service [10, 11].

The technical guidance for nosocomial infection control in this project featured: (a) Establishment of an appropriate system consisting of surveillance and reporting; (b) Preparation of a nosocomial infection control manual; (c) Development of reference and teaching materials such as video tapes, posters, leaflets, etc.; (d) Organizing of training courses for hospital staff; (e) Fact-finding study of nosocomial infection.

1-3-2 After the SARS epidemic

After the outbreak of SARS, the government of Japan dispatched the Japan Medical Team for Disaster Relief (JMTDR) including three medical doctors to offer cooperation in SARS control (March 16- April1, 2003) primarily at BMH. The team put emphasis on guidance for nosocomial infection control, and provided protective attires, disinfectants, and artificial respirators. In close collaboration with WHO, a nosocomial infection control workshop was organized, and the Guidelines for SARS Infection Control were drafted.

2. Guangdong Province in China

2-1 General aspects of the SARS outbreak

The first case of SARS in the world is now believed to be a case of severe pneumonia detected at Foshan, Guangdong Province in China in November 2002 [6]. The first patient was an executive staff member of an agricultural cooperative, and the second was a cook at a restaurant where dishes containing the meet of wild animals were served. Later, the disease spread in the Guangdong area, and there were 305 cases of SARS in the middle of February 2003. At that time, however, this severe disease of unidentified cause was considered to be a type of pneumonia caused by Chlamydia. It was only in April that the real aspect of the SARS outbreak in Guangdong Province was clearly identified (Figure 2).

Effective action for SARS control in Guangdong Province was delayed, but after April, the government of Guangdong Province took intensive actions to carry out nosocomial infection control procedures and began to publicly announce the actual status of the SARS outbreaks. On April 8, 2003, it was announced that the cumulative number of SARS cases in the province was 1,213 including 44 deaths and three foreigners. It was also proclaimed that the SARS epidemic was beginning to subside. According to the information records at K Hospital in Guangzhou City,

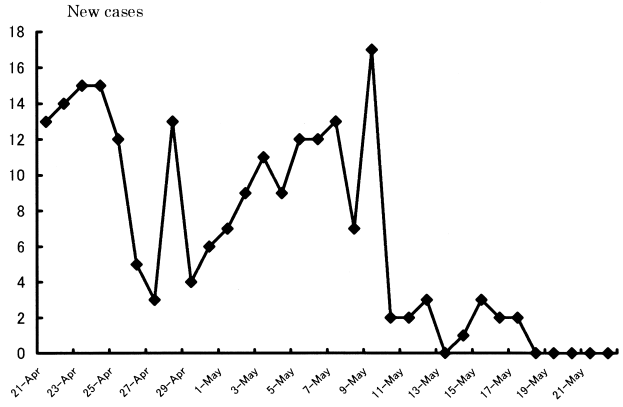

Figure 2. Occurrence of SARS in Guangdong Province, China. The first case occurred in November 2002, but the actual situation remained obscure for several months. After April 2003, SARS began to subside. The Medical Aid Team was dispatched from April 10 to 16 for health management of Japanese residents.

45 out of 83 cases with SARS had developed from nosocomial infection. After April, the number of the patients began to decrease and the epidemic came to a virtual end in late May.

2-2 Health management of Japanese residents

A Medical Aid Team including two doctors was dispatched to Guangdong Province to conduct health management against SARS among Japanese residents in the area and to eliminate anxiety. From April 10 to 16, 2003, the team visited the Japanese residents in major cities in Guangdong Province, where meetings and lectures were organized to provide an outline of SARS, preventive measures, and actions to be taken in emergency cases. Furthermore, up-to-date information on SARS and the medical situation was collected from the viewpoint of protecting Japanese residents. Since Guangdong Province was the area most intensively infected with SARS at that time, the Japanese residents (4,260 people) had very strong feelings of uneasiness and posed many questions, the most pressing of which was: “Which hospital should a patient visit when SARS-like symptoms appear? Is there any possibility that someone may be infected with SARS during a visit to a hospital? What actions should be taken if a suspected case appears at the working place?” [12].

3. Beijing

3-1 General aspects of the SARS outbreak

In China, SARS cases were detected in 21 provinces, two special administration cities and one autonomous region. Above all, the whole world was stuck with horror by the epidemic in Beijing. Shortly after the detection of the

first case on March 26, 2003, three cases of SARS were identified one after another. All of these patients were suspected to have been infected in Guangdong Province. In Beijing, the SARS infection spread further from these three cases. It is known that the first patient infected a total of 109 persons including hospital staff, family members, and relatives. At an early stage, there was no definite information on actual status of the SARS outbreak in Beijing. On April 20, the Chinese government announced a correction of the number of SARS cases, bringing it to a level 9 times as high as the initial announcement. It is known that nosocomial infection frequently occurred up to late April, accelerating the epidemic. According to a Xinhua News Agency announcement dated May 6, 2003, medical staff accounted for 25-30% of all patients with SARS. Thereafter, Chinese government and Beijing launched intensive efforts for effective control of SARS. From the middle of May, SARS infection began to subside in Beijing. On June 13, the number of new cases was zero, and on June 24, the designation of Beijing as a SARS infected area was repealed. The cumulative number of SARS cases in Beijing was 2,521 (Figure 3).

3-2 Actions taken for the control of SARS outbreak

From May, intensive actions for effective SARS control were carried out under the direction of the Ministry of Health of the Chinese Government and the Department of Health of Beijing City. Slogans to encourage the efforts for SARS control under close mutual cooperation appeared in many places in the city as well as in hospitals. A new system was established to officially commend medical staff who contributed to medical care for SARS patients. Eleven hospitals in the city were designated as hospitals specializing in SARS. Fever clinics and triage rooms were established in major hospitals. Efforts were made to promote nosocomial infection control, preparation of SARS control guidelines, disclosure of the information to the public, reinforcement of quarantine procedures, etc. Furthermore, collaboration with WHO and the Japanese government was started for SARS control.

In the hospitals designated for SARS control, drastic measures were taken for nosocomial infection control and the isolation of patients. Hospital staff devoted themselves to medical care of SARS cases and tried to acquire correct knowledge and techniques for nosocomial infection control. A 6-hour shift system (4 shifts per day) was introduced in hospitals. The staff in charge of SARS cases was not allowed to go beyond the compound of the hospital and boarding house during the service period of three weeks. After three weeks, the staff was ordered to receive a compulsory medical check-up and was placed under strict observation for the next two weeks. Big hospitals with 1,000 beds or more began to engage in medical care for SARS cases only. Putting emphasis on air ventilation, windows of patient rooms were kept open in most hospitals because negative pressure rooms were not always available. Strict infection control procedures were carried out for patient care, handling of specimens, waste disposal and laboratory examination (Photo. 2).

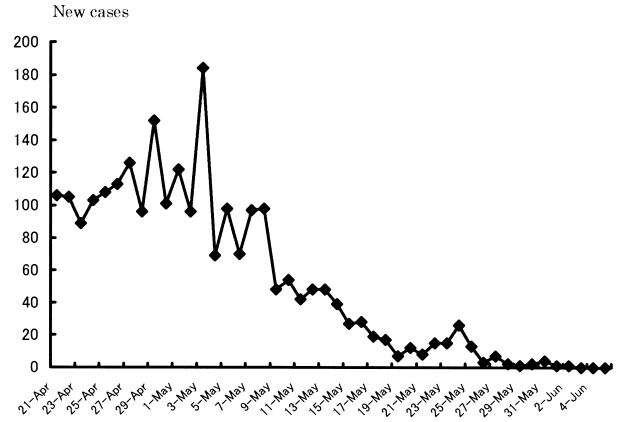

Figure 3. Occurrence of SARS cases in Beijing, China.
The first case occurred on March 26, 2003, but the actual situation remained unclear until April 20. Eleven hospitals including China-Japan Friendship Hospital were designated as SARS specializing hospitals and effective control measures were taken after May. The epidemic gradually subsided, and the designation of Beijing as a SARS infected area was repealed on June 24. The Japan Disaster Relief Team was dispatched from May 11 to 17.

3-3 Cooperation activities from Japan

To assist the SARS control activities in Beijing, JMTDR including two doctors was dispatched for a period

Photo. 2. Technicians in complete protective attire working in a laboratory exclusively set up for SARS cases (Courtesy of China-Japan Friendship Hospital).

from May 11 to 17, 2003. The team primarily offered cooperation activities at the China-Japan Friendship Hospital (CJFH). This hospital was founded under a grant-aid program from Japan in 1984 and has been functioning since then as one of the top referral hospitals in Beijing. The cooperation activities include: ① Technical guidance for nosocomial infection control, ② Supply of protective attires and artificial respirators and instruction on their use, ③ Technical guidance on nosocomial infection control.

Beijing City was faced with the most serious epidemic of SARS in the world. After the hospital began to introduce medical services as the most important SARS-specializing hospital (on May 8), 420 beds were allocated for the treatment of SARS cases (including 40 beds in ICU). An efficient system and SARS control measures were established, including: ① Establishment of a fever clinic and triage rooms, ② Zoning within the hospital according to infection risk in addition to improvements in wastewater treatment, ③ Improvement of air-conditioning and laundry facilities, ④ Organization of intensive training for nosocomial infection control and SARS treatment, ⑤ Formation of SARS treatment teams consisting of trained medical staff in the field of internal medicine, ICU, tracheotomy, X ray examination and laboratory examination.

In CJFH a small number of nosocomial infection cases occurred at the initial stage of the epidemic, but the zealous efforts of the hospital staff resulted in the implementation of proper infection control measures, and the hospital played a major role as a SARS-specializing hospital in Beijing without experiencing any more nosocomial infection cases. On May 15, a total of 167 SARS cases were under treatment at the hospital, which was the largest number in China.

DISCUSSION

During the SARS outbreak, a number of medical staff and patients' family members were infected through nosocomial infection [2, 4, 6]. Under such circumstances, Vietnam was the first country to succeed in SARS containment [8]. BMH made a particularly significant contribution to infection control after the middle of March when it took a major part in the treatment and control of SARS. The possible reasons for Vietnam's success in SARS control include: (1) Quick reaction and strong leadership of the Ministry of Health; (2) Implementation of adequate actions for nosocomial infection control; (3) Positive acceptance of support and assistance from WHO and foreign countries; (4) Disclosure of correct information on the disease; (5) Isolation of the patients to a single place; (6) Strict execution of quarantine procedures; (7) Ardent efforts of the staff of the Ministry of Health and hospitals.

The technical guidance given under the project, which had been introduced in the Bach Mai Hospital Project for Functional Enhancement before the SARS outbreak, also seemed to have made a contribution to the successful SARS control. When the first case of SARS was detected, the level of technical ability for nosocomial infection control among the hospital staff at BMH was considerably high. As a result, the techniques and knowledge acquired through training were quickly applied to clinical practice, followed by adequate actions for effective control of SARS [11]. It can be said that the accumulation of three years of solid sober training for hospital staff along with efforts to establish a nosocomial infection control system helped to lead BMH to these excellent results.

In the early stage of the SARS epidemic, China failed to adopt these actions ((1) - (7)) and was slow in disclosing correct information, resulting in frequent occurrence of nosocomial infection and spreading of the epidemic. However, after making enormous efforts to overcome the problems, China finally succeeded in effective control of SARS. In China, Taiwan, Hongkong, Canada and other countries, where many SARS cases occurred, adequate measures were delayed in the early stage of the SARS epidemic, and this may have resulted in the frequent occurrence of nosocomial infection. However, strong and proper actions were subsequently taken in these countries, eventually bringing SARS under control.

BMH in Vietnam and CJFH in China were the major hospitals supported by JMTDR from Japan. These hospitals were established under grant-aid projects from Japan followed by implementation of technical cooperation projects. Currently, these hospitals are making great contributions to medical care of the people and serving as symbols of friendship with Japan. The hospital staff received the present author with warm affection and enthusiasm. Successful collaboration focusing on nosocomial infection control and respiratory management could be deployed on the basis of firm reliance and the confidence of the staff created through the technical cooperation project in addition to the timely dispatch of our teams. The health management activities conducted in Guangdong Province also seem to have been timely and effective in terms of relieving anxiety and promoting preventive measures among Japanese residents.

Nosocomial infection control is one of the most important factors in providing high-quality medical care at the hospital level. First, special emphasis must be placed on observance of basic techniques (standard precautions) such as hand washing and the wearing of masks. Also, enlightenment activities, such as distribution of manuals and teaching materials and organizing of training courses for medical staff, are very useful and effective in improving nosocomial

infection control. In addition, as the actions for infection control of SARS, it is essential to take adequate measures against droplet infection, contagion and possible aerial infection [13, 14]. It is very important to train medical staff to master basic techniques and establish a control system at ordinary times, not just after the outbreak of an epidemic. Such a basis will make it possible to apply stringent nosocomial infection control promptly when outbreaks of SARS or other emerging diseases occur.

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REFERENCES

[1] Lee N, Hui D, Wu A, Chan P, Cameron P, Joynt GM. A major outbreak of severe acute respiratory syndrome in Hong Kong. New Engl J Med. 2003 May 3; 1-9.

[2] Poutanen SM, Low DE, Henry B, Finkelstein S, Rose D, Green K. Identification of severe acute respiratory syndrome in Canada. New Engl J Med. 2003; 348 (20): 1995 -2005.

[3] World Health Organization. Acute respiratory syndrome in China, Hong Kong Special Administrative Region of China, and Viet Nam. Weekly Epidemiol Record, WHO. 2003; 78: 73-74.

[4] Tsang KW, Ho PL, Ooi GC, Yee WK, Wang T, and Chan-Yeung M. A cluster of cases of severe acute respiratory syndrome in Hong Kong. New Engl J Med. 2003; 248 (29): 1977-1985.

[5] World Health Organization. Severe Acute Respiratory Syndrome (SARS). Weekly Epidemiol Record, WHO. 2003; 78: 81-83.

[6] World Health Organization. Severe Acute Respiratory Syndrome (SARS) in China and status of scientific and clinical knowledge. Weekly Epidemiol Record, WHO. 2003; 78: 129-136.

[7] Vu TH, Cabau JF, Nguyen NT, Lenoir M. SARS in Northern Vietnam. N Engl J Med. 2003; 348 (21): 2035.

[8] World Health Organization. Viet Nam SARS-free. Wkly Epidemiol Rec, WHO. 2003; 78: 145-46.

[9] Ohara H, Ikari K. Introduction of the community health direction in tertiary medical institutions- a trial in the Bach Mai Hospital Project for Functional Enhancement in Vietnam. Technology and Development. 2002; 16: 32-38.

[10] Ohara H. Technical guidance on nosocomial infection control in a hospital project in Vietam. The 44th General assembly on the Japanese Society of Tropical Medicine. 2002 November; Kochi, Japan. (in Japanese)

[11] Ohara H, Tateno S. Nosocomial infection control in Bach Mai Hospital Project in Vietnam and Application to SARS control. Kokusai Kyoryoku Kenkyu. 2003; 19 (2): 31-39 (in Japanese).

[12] Kirikae T, Ohara H. Report of the Medical Aid Team for Guangdong Province in China, health management of the Japanese against SARS. Tokyo: International Medical Center of Japan, 2003. (in Japanese)

[13] Li TST, Buckley TA, Yap FH. Severe acute respiratory syndrome (SARS): infection control. Lancet. 2003; 361: 1386.

[14] Seto WH, Tsang D, Yung RWH, Ching TY, Ng TK, Ho M, Ho LM, and Peiris JSM. Effectiveness of precautions against droplets and contact in prevention of nosocomial transmission of severe acute respiratory syndrome (SARS). Lancet, 2003; 361: 1519-1520.

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A METHOD FOR BLOOD EXAMINATIONS IN OVERSEAS MOBILE CLINICS: CLINICAL APPLICATION IN THE RURAL AREAS OF LAO PDR

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Abstract: We conducted an overseas mobile clinic in Lao PDR and examined the results of biochemical tests and blood examinations. For the biochemical tests, we used a method involving plasma skimming film and filter paper, called "plasma separation plate." In this study, we report the usefulness of the plasma separation plate, which is a newly developed method for the screening of biochemical tests in Japan. We were able to apply this method in a mobile clinic in Lao PDR. The quantitative concentration of plasma from blood collected from pricked fingers was measured by this method. Using the plasma skimming film and filter paper, the non-cell components in the blood were separated, and a quantity of the plasma was adsorbed by filter paper. Neither elaborate equipment nor electricity was required. The results of our test data indicated that few inhabitants suffered from adult diseases for which we were screening. We concluded, therefore, that this method is suitable for use in mobile clinics in the rural areas of developing countries.
Key words: plasma skimming film, mobile clinic, biochemical tests, Lao PDR, rural areas

INTRODUCTION

Until now, the results of biochemical tests conducted in mobile clinics in the rural areas of developing countries have been very difficult to review because centrifugal separation and preservation are not easy and require multiple pieces of equipment. The purpose of this study was to use plasma skimming film and filter paper, a newly developed method for the screening of biochemical tests called "plasma separation plate," and to apply this method in the mobile clinics of rural areas in Lao PDR.

MATERIALS AND METHODS

We conducted an overseas mobile clinic (medical checkup for inhabitants) in DakEuy village, SeKong province, Lao PDR from April 24 to May 16 (21 days) in 2002. Blood was collected on the basis of informed consent from the inhabitants of DakEuy village (168 subjects among 210 inhabitants: 0-5 years old, 26 people; 6-15 years old, 57 people; 16-65 years old, 85 people; 83 men and 85 women). This village is located near the Sekaman River, the rice fields cultivated by the villagers stretching along the riverbank. It is about 8 km from the Dakchung district town, which has a market that the villagers access by a day trip on foot. The village is about 20 km from the border of Vietnam. Most villagers travel on foot because there is no public transportation.

We conducted biochemical tests and blood examinations. The plasma separation plate was used for the biochemical tests. The plasma from blood collected by finger pricking was measured quantitatively using this method (Figure 1). We were able to examine the following parameters using the plasma separation plate: GOT (AST), GPT (ALT), γ-GTP, BUN, creatinine (Cr), total cholesterol (T-CHO), HDL-cholesterol (HDL-CHO), triglyceride (TG), amylase (AMY), uric acid (UA), and hemoglobin (Hb). HBs Ag and HCV Ab were examined using dry blood samples spotted on filter paper [1, 2]. Blood sugar (BS) was measured by the rapid blood glucose level monitor (Glutest sensor™, Sanwa Kagaku Kenkyusho Co. Japan) [3]. The data were confirmed to assure the accuracy of this method, and a storage stability of 3 days was also confirmed by drying plasma samples that had been absorbed on the filter paper in the plasma separation plate at room temperature. Furthermore, a temperature of 50℃ for 12 hours was confirmed for storage stability (Ishizuka *et al*., 2001) [4].

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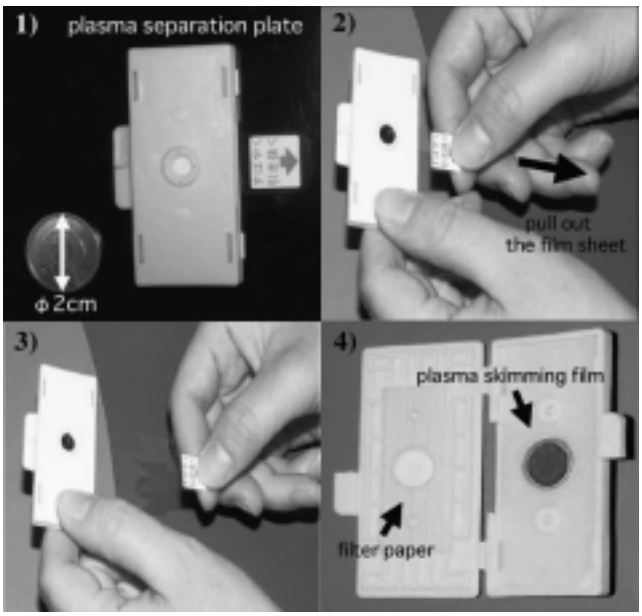

Figure 1 Plasma separation plate method

1）Four drops of blood are inserted into a hole in the middle of the opening on the surface of the plasma separation plate, and a small quantity of blood accumulates until a sufficient amount is obtained.
2）The storage is terminated by pulling out the film sheet.
3）The blood is left for 5 minutes after removal of the film sheet. Only the non-cell component is allowed to pass through the plasma skimming film, and the filter paper is allowed to adsorb it.
4）By opening the equipment, the conversion of the cell component in the blood is prevented (right arrow). For about 1 hour, the degradation of the component adsorbed in the filter paper (left arrow) is prevented by the aeration of the filter paper.

RESULTS

The results of the examinations were GOT (AST): 35.5 ± 16.7 (8～38 IU/l), GPT (ALT): 31.7 ± 10.3 (4～44 IU/l), γ-GTP: 35.5 ± 14.1 (16～73 IU/l), BUN: 11.5 ± 3.4 (8～20 IU/l), Cr: 0.4 ± 0.1 (0.4～1.1 mg/dl), T-CHO: 135.1 ± 21.2 (130～230 mg/dl), HDL-CHO: 31.7 ± 6.4 (35～88 mg/dl), TG: 107.9 ± 45.6 (50～130 mg/dl), AMY: 113.4 ± 82.0 (43～116 IU/l), UA: 3.3 ± 0.9 (2.5～8.3 mg/dl), Hb: 16.1 ± 1.8 (12.0～18.0 g/dl), BS: 88.6 ± 13.6 (70～110 mg/dl): Values are means ± SD (normal limit). From the results of the superscription, some inhabitants showed a higher than normal level of transaminase. Twelve persons (7 adults and 5 children) showed a higher than normal level of both GPT and GOT. The other results of the medical checkup were as follows: 7 cases of cold symptoms, 6 cases of ascariasis, and 1 case of suspected Behçet disease. In 4 cases of liver dysfunction, we measured HBs Ag and HCV Ab. Two cases were positive for HCV Ab, but none was positive for HBs Ag. Two women (one 40 and one 30 years old) were HCV Ab positive. The other cases were a 9-year-old girl and a 7-year-old boy who had cold symptoms. Some inhabitants showed higher than normal amylase levels. Six persons (3 adults and 3 children) showed levels over 200 IU/l. The breakdown was as follows: 3 cases of cold symptoms, 2 cases of ascariasis, 1 case of suspected Behçet disease. None of the subjects showed a higher than normal (upper range) for total cholesterol, uric acid or renal function. However, some inhabitants showed higher than normal triglyceride levels. Also, although the inhabitants were sufficiently nourished, no cases of diabetes mellitus were diagnosed. In DakEuy village, the hemoglobin values of inhabitants tended to be high but were within normal limits.

DISCUSSION

In this study, we used the plasma separation plate (mailing medical checkup kit), a method newly developed by the Aichi Medical Foundation of Diagnostic Technology, a co-author of this paper. Using plasma skimming film, the non-cell components in the blood are separated, and a certain quantity of the plasma is adsorbed by the filter paper. The specimens are transferred by mail and stored at room temperature. A multiphasic health screening method that utilizes convenience stores and the mailing of specimens is established in Japan [5]. With this method, blood can be collected by a prick of the finger and mailed to the Aichi Medical Foundation of Diagnostic Technology, thus making it possible for examinees to undergo the examination at home. The method does not require a centrifugal separator or power supply. Also, the sample, even when large, is light in weight and not bulky. This method is also useful for mobile clinics in the rural areas of developing countries because there is no need for elaborate equipment. Furthermore, the blood is not taken from the vein, making collection easy. There is also the advantage that the burden of the inhabitants is lowered because only small samples are required and the fear of injection is relieved [6, 7]. In this study, no inhabitant showed a higher than normal level of total cholesterol, uric acid or renal function. No diabetes mellitus patients were diagnosed. These findings indicated that few inhabitants suffered from adult diseases [8]. From this fact, we concluded that either the caloric intake of the inhabitants is comparatively low or that there are small groups who show hyperglycemia. Among the inhabitants who showed a higher than normal level of transaminase, amylase, and triglyceride, however, other factors such as meals, intake of alcohol, and viral or parasitic infection may be involved. We intend to further investigate this situation in the near future [9, 10]. In DakEuy village, the hemoglobin values of inhabitants tended to be high but within normal limits, perhaps because this village is located at an alti-

tude of over 1000 m [11]. We concluded that this method is suitable for the mobile clinics in rural areas of developing countries. Our results may contribute to the future development of medical checkups in rural areas without access to electricity.

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REFERENCES

1) Miyake C, Ueda M, Tsuki S, Kasugai T, Sakamoto N: Method of HbA_{1c} using dry blood sample spotted on filter paper -Application to medical examination for diabetes mellitus by mail -. J Japan Diab Soc 1997; 40: 791-4.
2) Itoh M, Gunawardena NK, Qiu XG, Weerasooriya MV, Kimura E: The use of whole blood absorbed on filter paper to detect Wuchereria bancrofti circulating antigen. Trans R Soc Trop Med Hyg 1998; 92 (5): 513-5.
3) Campbell EM, Redman S, Dunkley PR, Moffitt PS: The use of portable blood glucose monitors by trained lay operators. Med J Aust 1992; 157 (7): 446-8.
4) Ishizuka H, Miyake C, Ueda M, Tsuki S, Kasugai T, Ohta K: Evaluation of the biochemical examinations using the plasma skimming film -Application to medical examination by mail -. JMHTS 2001; 29 Suppl 1: 247.
5) Kasugai T, Ueda M, Tsuki S, Ohta K, Kobayashi S, Shirai M: Immnological fecal occult blood test by mail. Clin Gastroenterol 1994; 9: 407-17.
6) Brown SS: Laboratory support for rural health care. Proc R Soc Lond B Biol Sci 1980; 209 (1174): 119-28.
7) Perine PL, Nelson JW, Lewis JO, Liska S, Hunter EF, Larsen SA *et al*.: New technologies for use in the surveillance and control of yaws.
Rev Infect Dis 1985; 7: 295-9.
8) Khoo KL, Tan H, Liew YM, Deslypere JP, Janus E: Lipids and coronary heart disease in Asia. Atherosclerosis 2003; 169 (1): 1-10.
9) Bounlu K, Insisiengmay S, Vanthanouvong K, Saykham, Widjaja S, Iinuma K *et al*.: Acute jaundice in Vientian, Lao People's Democratic Republic. Clin Infect Dis 1998; 27 (4): 717-21.
10) Wu JC, Lee SD, Hsiao KJ, Wang SS, Chou P, Tsao D *et al*.: Mass screening of primary hepatocellular carcinoma by alpha-fetoprotein in a rural area of Taiwan—a dried blood spot method. Liver 1988; 8 (2): 100-4.
11) Heinicke K, Prommer N, Cajigal J, Viola T, Behn C, Schmidt W: Long-term exposure to intermittent hypoxia results in increased hemoglobin mass, reduced plasma volume, and elevated erythropoietin plasma levels in man. Eur J Appl Physiol 2003; 88 (6): 535-43.

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ANTIBIOTIC SUSCEPTIBILITY AND ITS GENETIC ANALYSIS OF VIBRIO CHOLERAE NON-O1, NON-O139 FROM ENVIRONMENTAL SOURCES IN LAO PEOPLE'S DEMOCRATIC REPUBLIC

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Abstract: In order to determine the epidemiological features of cholera in Lao PDR, the presence of mobile genetic elements such as plasmid, class I integron and SXT element in *V. cholerae* isolated from surface water were examined. Among the 22 strains isolated from 13 distantly separated sampling sites, no mobile genetic elements associated with drug resistance were found reflecting the antibiogram of the strains. Nevertheless, cholera epidemics due to multiple drug resistant *V. cholerae* occurred repeatedly in those areas until 2000.

Vibrio cholerae is a natural inhabitant of aquatic environments and is known to be the causative agent of the acute gastrointestinal disease. Transmission of *V. cholerae* is associated with consumption of contaminated water and contaminated foods [1, 2]. In spite of the importance and success of rehydration therapy for cholera, antimicrobial therapy still plays a critical role because it can reduce the volume of diarrhea as well as shorten the duration of symptoms and the excretion of *Vibrios* in the stool [3]. The occurrence of antibiotic-resistant strains of *V. cholerae* is being reported with increasing frequency [2, 4, 5]. However, the antibiotic susceptibilities of organisms spatially and temporally fluctuate. These susceptibilities have to be examined in order to achieve a better understanding of the epidemiological features.

In *V. cholerae*, antibiotic-resistant genes have been found on transmissible plasmids [6-8]. Recently, in addition to plasmids, integron and SXT element have also been described as vehicles in the transport of resistance genes [9, 10]. The analysis of antibiotic resistance determinants on these vehicles is being used as an epidemiological tool.

In Lao People's Democratic Republic (Lao PDR), the antibiotic susceptibility pattern of *V. cholerae* O1 isolated from cholera patients had been monitored for 8 years (1993-2000) [11, 12]. Although the pathogens were sensitive to the therapeutic antibiotics as expected until 1996, the pattern of antibiotic susceptibility began to change in 1998 [11]. We found that strains isolated before 1997 were resistant to streptomycin and harbored class1integron, while strains isolated after 1997 were resistant to chloramphenicol, tetracycline, streptomycin and sulfamethoxazole-trimethoprim and harbored SXT element [13]. In this study, antibiotic susceptibility and the presence of plasmid, class I integron and SXT element were examined in *V. cholerae* strains isolated from surface water in order to determine the epidemiological features of *V. cholerae* in Lao PDR.

A total of 13 surface water samples from branches of the Mekong River were collected in February 2003. This water is used routinely by people living near the river. The sampling sites were located in cholera epidemic areas until 2000, but no cholera patient was found in 2001, 2002 and 2003. Isolation of *V. cholerae* strains from the samples was accomplished by a modification of the method of Wai and colleagues [14]. About 100 ml of each sample water was taken into two sterile bottles containing 100 ml of 2% alkaline peptone water (pH～9.0～). One bottle was supplemented with sodium pyruvate (final concentration at 0.1%) or catalase (final concentration at 50 U/ml) to stimulate viable-but-nonculturable (VBNC) *V. cholerae* O1. All samples were incubated for 24 h at 37℃, and then cultured on thiosulfate-citrate-bile salt-sucrose (TCBS) agar for selective isolation of *Vibrio* species. After 24 h incubation at 37℃, yellow colonies were extracted from each TCBS agar and identification of isolates was performed by biochemical tests. The colony-growth pattern of the sample cultured in the supplemented media showed no differences from that cultured in a plain alkaline peptone water, and *V. cholerae* O1 was not detected at all. O-antigen was determined by slide agglutination with polyvalent O1, mono-specific Ogawa-Inaba antisera and with specific anti-O139 antisera

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obtained commercially (Denka Seiken Co., Tokyo, Japan). A total of 22 strains of *V. cholerae* were isolated. All of these environmental *V. cholerae* isolates were non-O1, non-O139 serotypes, and cholera toxin non-productive.

The minimum inhibitory concentrations (MICs) of nine antibiotics were determined by agar dilution technique according to the specifications of NCCLS [15]. To examine the production of beta-lactamase by ampicillin resistance isolates, clavulanic acid was used at a constant concentration of 4 μg/ml in combination with ampicillin. The susceptibility profiles of *V. cholerae* isolates are given in Table 1. Of the 22 environmental isolates, two were resistant to polymyxin B alone; one was resistant to polymyxin B and ampicillin; and 15 were resistant to ampicillin alone. All isolates were highly sensitive to the other antibiotics examined. For all of the ampicillin resistant isolates, clavulanate reduced the level of MICs to the same as that for the susceptible isolates.

Since plasmids are known to encode resistance to beta-lactams, the presence of plasmid was examined by the modified method of Kado and Liu [16]. Cells were grown on nutrient agar plates overnight at 37℃. Colonies were scraped from the plates and suspended in 100 μl of a solution containing 50% glucose, 25 mM EDTA and 1 M Tris (pH 8.0). Then 200 μl of lysing solution containing 1% SDS and 0.2 N NaOH was added. After lysis, 300 μl of phenol-chloroform-isoamylalcohol (25: 24: 1) was added, and the samples were mixed well. After centrifugation, a sample of the supernatant was electrophoresed on a 0.5% Seakem ME agarose. *Shigella flexneri* 2a YSH 6000 [17] harboring approximately 230 kb plasmid was used as the control strain. None of the 16 ampicillin resistant isolates harbored any plasmids (data not shown).

Next, we investigated the presence of class I integron, which has been reported to contain a gene cassette that conferred resistance to beta-lactams [18]. To identify class I integron, primers inDS-F (5'-CGGAATGGCCGAGCAGATC-3') and inDS-B (5'-CAAGGTTTGGACCAGTTGCG-3'), which are specific for the 5'conserved segment containing integrase gene (*intI 1*) [19], were used to amplify this region by PCR. PCR with inDS-F and inDS-B primers did not yield amplicons for any of the 22 environmental isolates but yielded an 871 bp amplicon for the clinical isolate 93LA5 as a positive control (Fig. 1).

Table 1. Antibiotic susceptibilities of *V. cholerae* isolates

Strain*	MIC (μg/ml)§									*intI 1*	int_{SXT}
	PMX	TC	AMP	AMP-CLA	EM	NA	CP	ST	SM		
3p-4	0.4	0.8	>100	0.4	3.13	0.1	0.8	10.0	12.5	−	−
3p-7	>100	0.2	6.25	1.6	12.5	0.2	1.6	10.0	6.25	−	−
3p-8	0.4	0.2	>100	0.4	12.5	0.1	0.8	5.0	12.5	−	−
3p-9	0.4	0.2	>100	0.8	12.5	0.1	0.8	5.0	12.5	−	−
3p-10	0.4	0.2	>100	0.8	12.5	0.2	0.8	5.0	12.5	−	−
3p-11	0.4	0.2	>100	0.4	12.5	0.2	0.8	5.0	12.5	−	−
3p-13	0.4	0.2	>100	0.4	12.5	0.2	0.8	5.0	12.5	−	−
3p-14	0.4	0.2	>100	0.8	12.5	0.2	0.8	5.0	12.5	−	−
3p-15	>100	0.4	6.25	3.13	12.5	0.2	1.6	10.0	6.25	−	−
8p-2	0.8	0.4	>100	0.8	12.5	0.2	1.6	2.5	12.5	−	−
11pw--2	0.8	0.4	>100	0.8	12.5	0.2	1.6	2.5	12.5	−	−
12pw-2	3.13	0.4	>100	0.4	12.5	0.2	1.6	10.0	12.5	−	−
12pw-3	3.13	0.4	>100	0.8	6.25	0.2	1.6	5.0	12.5	−	−
12pw-4	3.13	0.4	>100	0.8	6.25	0.2	1.6	5.0	12.5	−	−
12pw-6	3.13	0.4	>100	0.8	6.25	0.2	0.8	10.0	12.5	−	−
12pw-7	3.13	0.4	>100	0.8	6.25	0.2	0.8	5.0	12.5	−	−
12cpw-2	1.6	0.2	>100	0.8	6.25	0.2	0.8	5.0	6.25	−	−
13pw-2	1.6	0.4	6.25	1.6	25	0.2	0.8	40.0	12.5	−	−
13pw-3	0.8	0.4	6.25	3.13	25	0.2	1.6	40.0	6.25	−	−
13pw-4	1.6	0.8	6.25	3.13	25	0.2	1.6	40.0	6.25	−	−
13pw-5	1.6	0.8	6.25	3.13	25	0.2	1.6	40.0	12.5	−	−
13pw-7	>100	0.8	>100	1.6	3.13	0.2	0.8	5.0	6.25	−	−

* p, strain recovered from peptone water with pyruvate; pw, strain recovered from peptone water without supplement; cpw, strain recovered from peptone water with catalase.

§ Abbreviations: PMX, polymyxin B; TC, tetracycline; AMP, ampicillin; CLA, clavulanic acid; EM, erythromycin; NA, nalidixic acid; CP, chloramphenicol; ST, sulfamethoxazol-trimethoprim; SM, streptomycin.

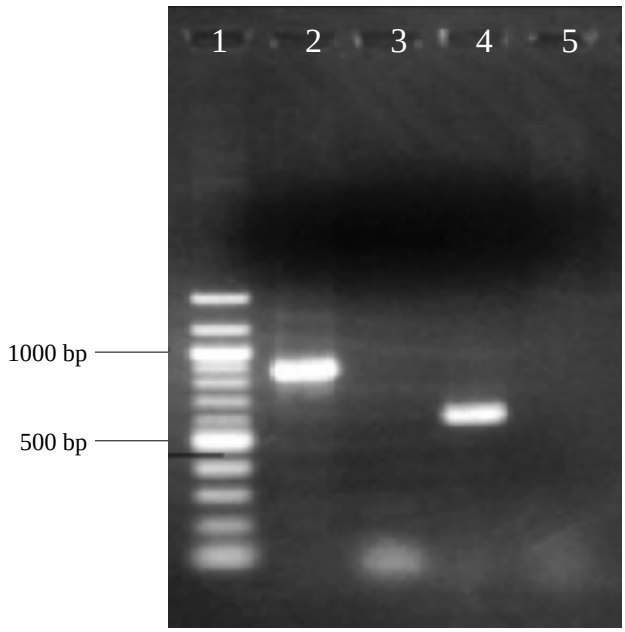

Fig. 1. Examples of PCR for the presence of class I integron using the primers inDS-F and inDS-B (lanes 2 and 3) and SXT element using primers INT1and INT2 (lanes 4 and 5) among *V. cholerae* non-O1, non-O139 strains isolated from Lao PDR. Lanes: 1,100-bp molecular mass standard; 2, strain 93LA5 (clinical isolate in 1993 harboring class I integron); 3 and 5, strain 3p-8 (environmental isolate); 4, strain 00LA1 (clinical isolate in 2000 harboring SXT element).

SXT element is responsible for resistance to chloramphenicol, streptomycin, sulfamethoxazole and trimethoprim in *V. cholerae* [20]. Recently Hochhut et al. reported that deletion of the antibiotic resistance genes located on SXT element rendered these strains sensitive to the antibiotics [20]. We also noticed that *V. cholerae* O1 harboring SXT element were sensitive to all antibiotics examined, and in these strains, antibiotic resistant gene cluster was absent from the SXT element. All of the 22 isolates were sensitive to these four antibiotics, but we analyzed the possible presence of the SXT element backbone by PCR. Primers INT1 (5'-GCTGGATAGGTTAAGGGCGG-3') and INT2 (5'-CTCTATGGGCACTGTCCACATTG-3'), which are specific for SXT integrase gene (int_{SXT}) [20], were used. However, the expected 592 bp amplicon was not obtained from any of the environmental isolates (Fig. 1).

V. cholerae O1 and O139 are known to enter a viable-but-non-culturable (VBNC) state in the environment, and recovery of the non-culturable cells from samples relies on culture methods [21]. In this study, all environmental isolates were non-O1, non-O139 serotype isolated from samples by treatment with catalase or sodium pyruvate which enhance the recovery of non-culturable cells [14]. The failure to detect *V. cholerae* O1 may have been due to their small population among a large number of non-O1 Vibrios. Therefore, further trials, such as immuno-magnetic separation technique using antibody against *V. cholerae* O1, are recommended.

The environmental non-O1, non-O139 isolates in this study showed resistance to polymyxin B and/or ampicillin. Resistance to these antibiotics was found to be common among the environmental non-O1, non-O139 isolates from Malaysia, Thailand and South India [2, 22, 23]. This result illustrates the occurrence of antibiotic-resistant environmental non-O1, non-O139 *V. cholerae* strains worldwide. However, it should be noted that environmental non-O1, non-O139 isolates clearly showed resistance to fewer antibiotics in comparison with clinical *V. cholerae* O1 isolates from Lao PDR [13]. In addition, there was a clear difference in distribution of class I integron and SXT element among clinical O1 isolates (unpublished observations) and environmental non-O1, non-O139 isolates from Lao PDR, as none of the environmental isolates harbored class I integron or SXT element. Thungapathra and colleagues [24] showed that multiple antibiotic resistances were more common among clinical isolates. Dalsgaard and colleagues reported that distribution of class I integron was associated with therapeutic use of antibiotics [25]. Thus, it seems unlikely that environmental non-O1, non-O139 isolates from Lao PDR are under antibiotic stress. In other words, the environmental waters examined are not contaminated with the organisms from a clinical origin.

The majority of ampicillin resistant *V. cholerae* contained plasmids and showed resistance to multiple antibiotics [8, 9, 23-25]. However, in Lao PDR, environmental isolates were resistant only to ampicillin without harboring plasmid, and *V. cholerae* O1 isolates from cholera patients were sensitive to ampicillin [12]. Class I integron and SXT element are occasionally lacking the drug resistance gene, but, they can acquire the resistance gene later. Therefore, Class I integron and SXT element should be monitored even in drug susceptible *V. cholerae*.

In the background of the present study, there is a fact that *V. cholerae* O1 in the world is simultaneously gaining resistant to a variety of anti-microbial drugs. There is a possibility that the transmission of SXT element takes place in the environmental water where *V. cholerae* consists of a flora, but this seems unlikely because we failed to find SXT element in the environmental *V. cholerae*. The transmission route of SXT element remains unclear.

REFERENCES

1. Pitrak DL, Gindorf JD. Bacteremic cellulites caused by

non-O1 *Vibrio cholerae* acquired in a freshwater inland lake. J Clin Microbiol 1989; 27: 2874-6.
2. Radu S, Ho YK, Lihan S, et al. Molecular characterization of *Vibrio cholerae* O1 and non-O1 from human and environment sources in Malaysia. Epidimiol Infect 1999; 123: 225-32.
3. Mahalanabis D, Molla AM, Sack DA. Clinical management of Cholera. In: Barua D, Greenough WB III, eds. Chorela. New York: Plenum Medical, 1992; 253-83.
4. Bag PK, Maiti S, Sharma C, et al. Rapid spread of the new clone of *Vibrio cholerae* O1 biotype El Tor in cholera endemic areas in India. Epidemiol Infect 1998; 121: 245-51.
5. Dalsgaard, A., Forslund, A., Bodhidatta, L., et al. A high proportion of *Vibrio cholerae* strains isolated from children with diarrhea in Bangkok, Thailand are multiple antibiotic resistant and belong to heterogeneous non-O1, non-O139 O-serotypes. Epidemiol Infect 1999; 122: 217-26.
6. Dupont M. J., Jouvenot M, Couetdic G, Michel-Briand Y. Development of plasmid-mediated resistance in *Vibrio cholerae* during treatment with trimethoprim-sulfamethoxazole. Antimicrob Agents Chemother 1985; 27: 280-1.
7. Reid AJ, Amyes GB. Plasmid penicillin resistance in *Vibrio cholerae*: identification of new β-lactamase SAR-1. Antimicrob Agents Chemother 1986; 30: 245-7.
8. Young H, Nandivada LS, Amyes GB. Antibiotic resistance in the tropics. 1. The genetics bacterial ampicillin resistance in tropical areas. Trans R Soc Trop Med Hyg 1989; 83: 38-41.
9. Dalsgaard A, Forslund A, Tam NV, Vinh DX, Cam PD. Cholera in Vietnam: changes in genotypes and emergence of class I integrons containing aminoglycoside resistance gene cassettes in *Vibrio cholerae* O1 strains isolated from 1979 to 1996. J Clin Microbiol 1999; 37: 734-41.
10. Waldor MK, Tschäpe H, Mekalanos JJ. A new type of conjugative transposon encodes resistance to sulfamethoxazole, trimethoprim, and streptomycin in *Vibrio cholerae* O139. J Bacteriol 1996; 178: 4157-65.
11. Higa N, Iwanaga M, Utsunomiya A, et al. Drug sensitivity *of Vibrio cholerae* and *Shigella* species in the world. Jpn J Trop Med Hyg 1995; 23: 159-64.
12. Phantouamath B, Sithivong N, Sisavath L, et al. Transition of drug susceptibilities of *Vibrio cholerae* O1 in Lao People's Democratic Republic. Southeast Asian J Trop Med Public Health 2001; 32: 95-9.
13. Iwanaga M, Toma C, Miyazato T, Insisiengmay S, Nakasone N, Ehara M. Antibiotic resistance conferred by a class I integron and SXT constin in *Vibrio cholerae* O1 strains isolated in Laos .Antimicrob Agents Chemother. 2004; 48: 2364-9.
14. Wai SN, Mizunoe Y, Takeda A, Yoshida S. A comparison of solid and liquid media for resuscitation of starvation- and low-temperature-induced nonculturable cells of *Aeromonas hydrophila*. Arch Microbiol 2000; 173: 307-10.
15. National Committee for Clinical Laboratory Standards. 2001. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 5th ed., Vol.20, no.2. Approved standerd M7-A5. National Committee for Clinical Laboratory Standers.
16. Kado CI, Liu ST. Rapid procedure for detection and isolation of large and small plasmids. J Bacteriol 1981; 145: 1365-73.
17. Sasakawa C, Kamata K, Sakaki T, Murayama SY, Makino S, Yishikawa M. Molecular alteration of the 140-megadalton plasmid associated with loss of virulence and congo red binding activity in *Shigella flexneri*. Infect Immun 1986; 51: 470-5
18. Mazel D, Davies J. Antibiotic resistance in microbes. Cell Mol Life Sci 1999; 56: 742-54.
19. Dalsgaard A, Forslund A, Sandvang D, Arntzen L, Keddy K. *Vibrio cholerae* O1 outbreak isolates in Mozambique and South Africa in 1998 are multiple-drug resistant, contain the SXT element and the aadA2 gene located on class I integrons. J Antimicrob chemother 2001; 48: 827-38.
20. Hochhut B, Lotfi Y, Mazel D, Faruque SM, Woodgate R, Waldor MK. Molecular analysis of antibiotic resistance gene cluster in *Vibrio cholerae* O139 and O1 SXT constins. Antimicrob Agents Chemother 2001; 45: 2991-3000.
21. Colwell RR, Brayton PR, Grimes DJ, Roszak DB, Huq SA, Palmer LM. Viable but non-culturable *Vibrio cholerae* and related pathogens in the environment: implication for release of genetically engineered microorganisms. Bio/Technology 1985; 3: 817-20.
22. Dalsgaard A, Serichantalergs O, Pitarangsi C, Echeverria P. Molecular characterization and antibiotic susceptibility of *Vibrio cholerae* non-O1. Epidemiol Infect 1995; 114: 51-63.
23. Thomson CJ, Jesudason MV, Balaji V, Malathi B, Mukundan U, Amyes SGB. The prevalence of *Vibrio* spp. in drinking water and environmental samples in Vellore South India. Epidemiol Infect 1998; 121: 67-76.
24. Thungapathra M, Amita, Sinha KK, et al. Occurrence of antibiotic resistance gene cassettes *aac (6')-Ib*, *dfrA5*, *dfrA12*, and *ereA2* in class I integrons in non-O1, non-O139 *Vibrio cholerae* strains in India. Antimicrob Agents Chemother 2002; 46: 2948-55.
25. Dalsgaard A, Forslund A, Serichantalergs O, Sandvang D. Distribution and content of class I integron in different *Vibrio cholerae* O-serotype strains isolated in Thailand. Antimicrob Agents Chemother 2000; 44: 1315-21.

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A SIMULATION SHEDS A LIGHT ON THE PRESENT HIV EPIDEMIC

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Abstract: A hypothetical community of one million people where birth rate and death rate are equal was applied to the modified deferential equations Lipsitch and Nowak published in 1995 in order to examine the impact of partner acquisition change on the HIV epidemic over a relatively short term. The results showed that if the partner exchange rate increases from two to three per year in the population, the epidemic caused by a more virulent strain would overweigh that caused by a less virulent strain within a century. This result reveals that an increase in the rate of partner acquisition gives the more virulent strain an advantage in terms of propagating the virus in a given population, at least over a relatively short term of several decades. The partner acquisition rate also exerts an influence on the magnitude of the HIV epidemic and the time it needs to reach a peak in the hypothetical community.

These results indicate that increased sexual contact may be even more important than expected and thus shed a new light on the present HIV epidemic.

INTRODUCTION

To maintain the infectious cycle, a sexually transmitted virus has to remain infectious until at least one member of a couple engages in sexual activity outside the pair. When a sexually transmitted virus, such as HIV-1, exists in a society of relatively monogamous humans, only those pathogens that have some way to prolong infectiousness can be maintained. In other words, to extend their infectiousness, pathogens must avoid both being destroyed by the host's immune system, [1] and causing the host to die. From the evolutionary standpoint, the benefits to the virus of extending infectiousness have to be weighed against both the higher rate of infection and shorter period of infectiousness that is seen in measles, influenza and many other pathogens [2-4]. If this hypothesis is true, what keeps the slowly replicating HIV from being displaced by the rapid replicators in the HIV population as a whole?

An increase in the number of sexual partners should give HIV a chance to spread from one host to another. Even if the high replication-type virus ruins the health of the human host within a relatively short period of time, and, as a result, shortens the period of infectiousness, this disadvantage would be compensated by the increasing chance of infection through sexual contacts.

This conjecture points to a key issue, that is, the fact that the higher the sexual partner rate is, the larger the net benefits to HIV-1from rapid replication will be.

Mathematical modeling promises to provide a powerful tool to evaluate this issue. In the present paper we look at this possibility, with special focus on how much impact an increase in the number of partners exerts on the competition of two different types of HIV-1, i.e. a virulent HIV and a less virulent HIV in the population.

MATERIALS AND METHODS

Mathematical modeling

There have been several studies using mathematical modeling to deal with theoretical predictions about the evolution of parasite virulence [5-7]. Among these, the paper by Lipsitch and Nowak provides important insights into the evolution of HIV-1, investigating whether greater transmission opportunities exist for shorter-duration-of infection strains [8].

We use this mathematical model to determine which strain becomes dominant, the virulent or less virulent strain, as the rate of partner change increases. The spread of the

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two strains was modeled on the basis of the differential equation created by Lipsitch and Nowak in 1995 [8]. One difference is that, whereas Lipsitch and Nowak applied exponential population growth, we simplify the model, applying a constant population in which birth rate is equal to death rate in order to determine the impact of a change in the acquired number of sexual partners on the competition of two different types of HIV, a virulent strain and less virulent strain, over a relatively short period of time.

The following system of differential equations is used:

$$dS/dt = u*N - (A_1 + A_2 + u)*S \quad (1)$$

$$dY_1/dt = A_1*S - m_1*Y_1 - u*Y_1 \quad (2)$$

$$dY_2/dt = A_2*S - m_2*Y_2 - u*Y_2 \quad (3)$$

Where S is the number of susceptible persons in the population, and Y_1 and Y_2 represent the number of hosts infected with strain 1 and 2, respectively. N is the total population size. New susceptibles are born to all members of the population at a rate of u. Given the prerequisite that population is constant, meaning that birthrate is equal to death rate, u is also the rate at which the hypothetical population dies. The m_i is given by mortality caused by HIV.

The A_i is the so-called "force of infection" for strain i, which is expressed by the following equation:

$$A_i = c*b_i*Y_i/N \quad (4)$$

where c is the rate of new partner acquisition per year, and bi is the probability that a susceptible person will be infected with strain i when an infected individual encounters the susceptible partner.

Hypothetical community and HIV characteristics

The equations are tested under the hypothetical condition that a total population is one million, that life expectancy at birth of the population is fifty years (with an annual death rate of 2%), and that the period of time from infection to death is 3.3 years in the virulent strain and ten years in the less virulent strain. The probability of HIV infection per contact was estimated to be 0.01, which means that an infection occurred every 100 contacts.

RESULTS AND DISCUSSION

Interesting results were obtained from the equations and are shown in Figures 1-a, b, c, and d. Given that partner acquisition rate per year is one, if a single index case of the virulent strain (strain 1) and less virulent strain (strain 2) are introduced into the totally susceptible population of one million people, no epidemic will occur (Fig. 1-a). However, if the partner acquisition rate increases from one to two on average, the less virulent strain will spread over a couple of centuries and reach a stable status, maintaining a prevalence of about seven percent whereas the virulent strain will die out after a small epidemic (Fig. 1-b). If the partner exchange rates increase from two per year to three in the same population, the epidemic caused by the more virulent strain will overweigh that caused by the less virulent strain over several centuries (Fig. 1-c). As the number of partner acquisitions increases from three per year to four, the more virulent strain will become dominant in the early stage of the epidemic in the population, with a peak of over thirty percent of prevalence (Fig. 1-d). If the partner acquisition rate increases, the epidemic caused by the virulent strain will occur only within half a century (Fig. 1-d).

This result may provide an answer to the question of why HIV-1 has become pandemic within a few decades. Although the mathematical modeling is simple, the result reveals that an increase in the rate of partner acquisition gives the virulent strain an advantage in terms of propagating the virus in a given population, at least over a short period of time. It follows that a reduction in partner acquisition rate may contribute to preventing the HIV-1 epidemic both indirectly by depriving the virulent strain of its advantage of spreading in the population and directly by reducing the ability of all strains to transmit one after another.

Furthermore, as partner acquisition rate increases, the subsequent epidemic magnifies more rapidly and less time is needed for the HIV epidemic to reach a peak (Fig. 1-a, b, c, d). In our simulation, when the partner acquisition rate is four per year on average, the HIV epidemic reaches its peak in a mere 25 years, with more than 30% of the population infected with HIV (Fig. 1-d).

Studies from Uganda have revealed that HIV-1 prevalence among young adults significantly declined as a result of behavioral change since the early 1990s. [9-11]. Among women aged 15-19 years, education and marital status-adjusted HIV-1 prevalence declined from 32.2% in 1991 to 10.3% in 1997. For 20-24-year-old women, HIV-1 prevalence decreased from 31.7% in 1993 to 21.7% in 1997. In Uganda, falls in HIV-1 prevalence have been associated with a decrease in the number of sexual partners and pregnancy rate in teenagers, and an increase in reported age at first sex and the frequency of reported condom use. Social factors, such as frequent sexual contact, may be even more important than expected.

The mathematical modeling of Lipsitch and Nowak provided information about long-term historical evolution, giving the unit of time rather than actual time frame work. However, setting the hypothetical population and applying it to our simplification of the mathematical model tells us more intuitively what impact partner acquisition change exerts on the HIV epidemic in a relatively short period of time,

Figure 1-a

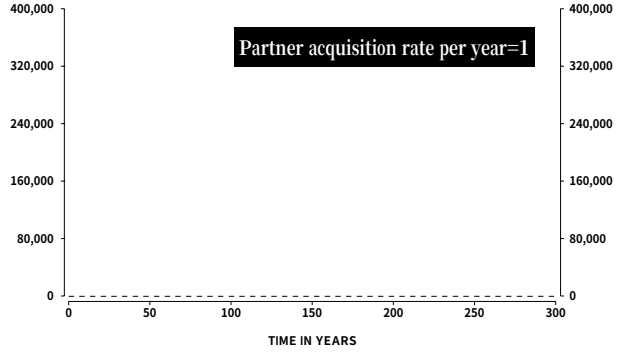

Figure 1-b

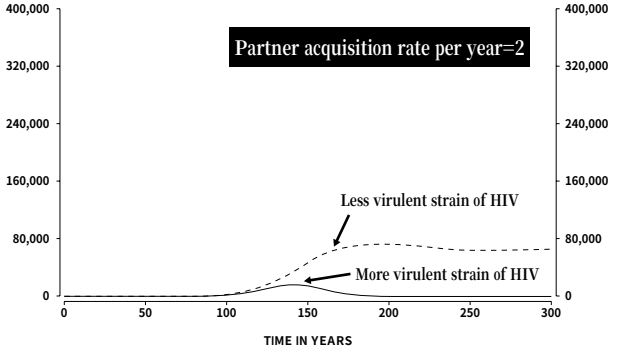

Figure 1-c

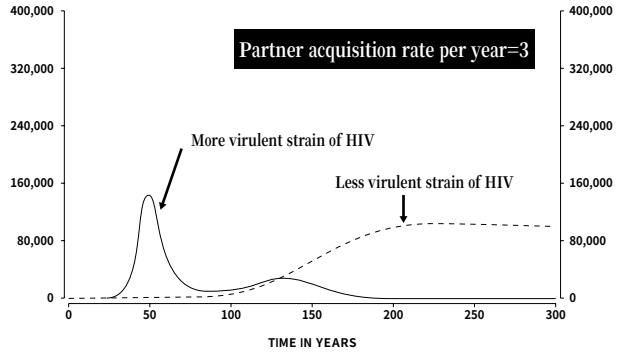

Figure 1-d

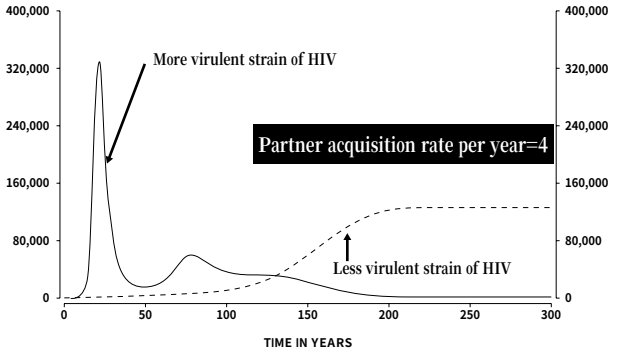

Fig. 1-a, b, c, and d.
The possible dynamical patterns in competition between two strains in a host population are shown. The x axis shows the year. The y axis is the number of individuals infected with the less virulent strain and those infected with the virulent strain. The more frequently people intermingle, the more dominant the more virulent strain will be.

i.e. several decades. Because Lipsitch and Nowak set the unit of time rather than actual time frame, or year, their model provides information about the HIV epidemic both in the short-term and long term but does not reveal the exact meaning of these time frames.

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REFERENCES

1. Ewald, P.W., *Evolution of Infectious Disease.* 1994, New York: Oxford Univ Press.
2. Fenner, F., *Infectious disease and social change. I.* Med J Aust, 1971. 1 (20): p.1043-7.
3. Clements, C.J. and F.T. Cutts, *The epidemiology of measles: thirty years of vaccination.* Curr Top Microbiol Immunol, 1995. 191: p.13-33.
4. McLean, A.R., *After the honeymoon in measles control.* Lancet, 1995. 345 (8945): p.272.
5. Levin, B.R. and J.J. Bull, *Short-sighted evolution and the virulence of pathogenic microorganisms.* Trends Microbiol, 1994. 2 (3): p.76-81.
6. May, R.M. and R.M. Anderson, *Epidemiology and genetics in the coevolution of parasites and hosts.* Proc R Soc Lond B Biol Sci, 1983. 219 (1216): p.281-313.
7. Ewald, P.W., *Transmission modes and the evolution of virulence: with special reference to cholera, influenza, and AIDS.* Human Nature, 1991. 2: p.1-30.
8. Lipsitch, M. and M.A. Nowak, *The evolution of virulence in sexually transmitted HIV/AIDS.* J Theor Biol, 1995. 174 (4): p.427-40.
9. Kamali, A., et al., *Seven-year trends in HIV-1 infection rates, and changes in sexual behaviour, among adults in rural Uganda.* Aids, 2000. 14 (4): p.427-34.
10. Mulder, D., et al., *Decreasing HIV-1 seroprevalence in young adults in a rural Ugandan cohort.* Bmj, 1995. 311 (7009): p.833-6.
11. Asiimwe-Okiror, G., et al., *Change in sexual behaviour and decline in HIV infection among young pregnant women in urban Uganda.* Aids, 1997. 11 (14): p.1757-63.