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EMERGING DISEASES IN INDONESIA: CONTROL AND CHALLENGES

I NYOMAN KANDUN
Recieved 31, October, 2006

ABSTRACT: Infectious diseases remain an important cause of morbidity and mortality in Indonesia. The reduction, elimination, and eradication of infectious diseases have been the subject of numerous meetings and public health initiatives for decades. The malaria, yaws and other communicable disease eradication programs of earlier years, although unsuccessful, contributed greatly to an understanding of the difficulties faced in trying to achieve goal of disease control. The reemergence of old infectious diseases, along with the emergence of new diseases such as SARS, Avian Influenza and the development of antimicrobial resistance, pose significant challenges to public health.

BACKGROUND

The control, elimination and eradication of human diseases have been the subject of numerous public health interventions and discussions for decades. The eradication of smallpox was declared on May 8, 1980 at the 33rd World Health Assembly, and was followed soon after by poliomyelitis and other eradicable diseases. Although many diseases eradication program in the past were unsuccessful, the lessons learnt contributed to an understanding about the complexities and difficulties faced in trying to achieve the ultimate goal of disease control. An understanding of the natural history of a disease, including multiple causation and biological, sociopolitical and economic issues sheds light on the public health interventions available in dealing with communicable diseases and their containment.

Indonesia stretches from west to east, with an area of 1.9 million $km^2$and a population of 230 million. In developing countries like Indonesia, health resources have always been limited, and decisions as to the most preferable and cost effective intervention programs have to be targeted to priority diseases. Disease control can be defined as the reduction of diseases in a defined geographical area as a result of deliberate control efforts. This definition should be further quantified to indicate the level of disease reduction to be achieved. Disease elimination and eradication are the ultimate goals of any public health intervention starting from disease control. Communicable diseases are still serious public health problems, killing and causing suffering for millions of people in Indonesia, especially the most vulnerable groups, i.e. the poor, women and children. Communicable diseases exert a negative effect on development and place a burden on the economy of the individual and the country as a result of the huge costs of treatment and control.

Technical solutions combined with strategies to mobilize all levels of society from high level decisions-makers to communities and families will ensure the effective control and prevention of communicable diseases. The following is a brief description of re-emerging diseases, newly emerging diseases, and the challenges encountered in their control.

DENGUE HEMORRHAGIC FEVER

An epidemic of dengue fever / dengue hemorrhagic fever (DF/DHF) started in the Southeast Asia region after the Second World War. The first case of DF/DHF in Indonesia was reported in 1968 from Jakarta and Surabaya. Since then the frequency and magnitude of DF/DHF outbreaks have increased dramatically. As the principal mosquito vector, Aedes aegypti and the viruses (D_1, D_2, D_3, D_4) that cause DF /DHF, expanded their geographical and age distribution nationally. Figure 1 presents the distribution of DHF by province while Figure 2 presents the seasonal variation of DHF cases and deaths in 2004－2005 and a comparison of DHF cases in 2005 and 2006.

The epidemiologic trends in recent decades demonstrate that the prevention and control of dengue virus transmission have failed. There is no vaccine available for dengue viruses nor effective mosquito control programs dealing with breeding places. Emphasis has been placed on disease surveillance and immediate response using space sprays targeting adult mosquitoes in the affected focal areas. Now

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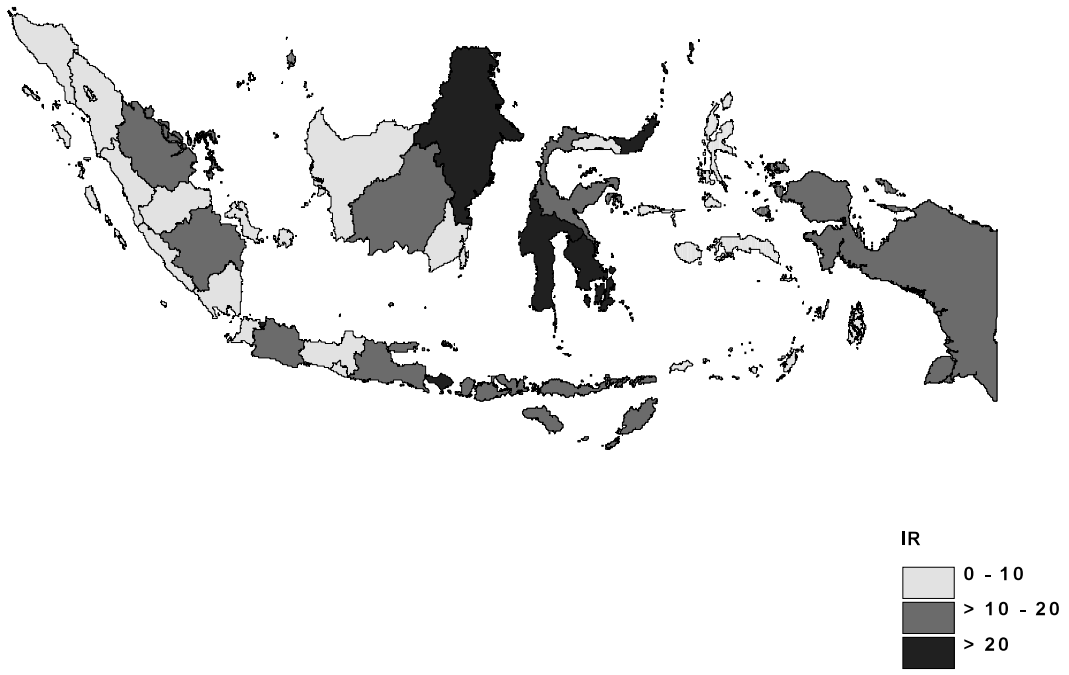
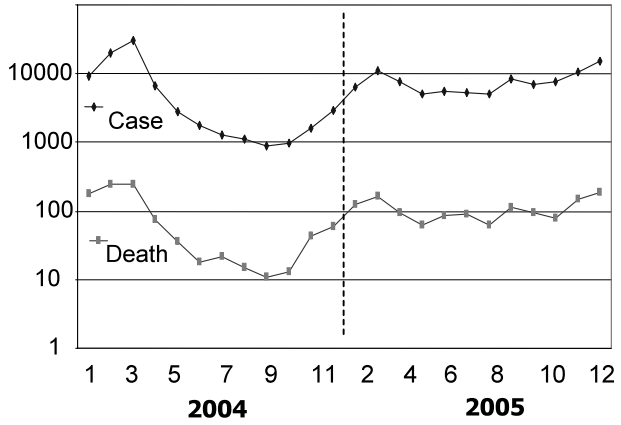


Figure 1. INCIDENCE OF DENGUE HEMORRHAGIC FEVER (DHF) BY PROVINCE IN 2005

a. Trends of Dengue Hemorrhagic Fever Cases & Deaths in 2004-2005



b. NUMBER OF DENGUE HEMORRHAGIC FEVER CASE IN 2005-2006 (UP TO 19 June 2006)

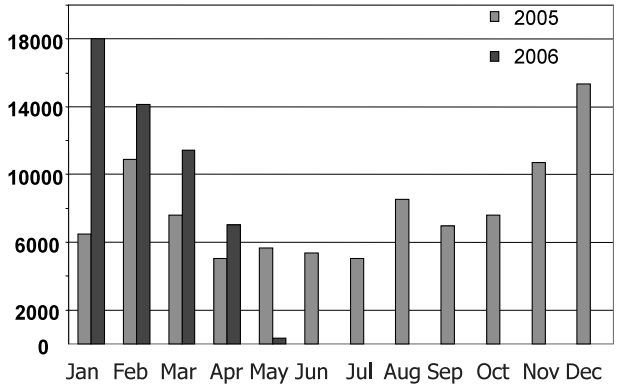


Figure 2.

strategies are being revised so as to focus on community involvement in the elimination of breeding places and to improve partnership and professionalism among program managers at all levels.

A decentralized integrated approach that targets larval mosquitoes is being implemented for effective Aedes aegypti control. Along with high-level political commitment, community involvement is an important prerequisite for vector control.

TUBERCULOSIS (TB)

Tuberculosis (TB) is a bacterial disease caused by Mycobacterium tuberculosis transmitted primarily by airborne droplets. Infection occurs when susceptible persons inhale infected droplets produces by coughs and sneezes of persons with active lung TB. TB causes suffering for millions of people, particularly the poor, women, children, and HIV/AIDS patients. More than half a million new TB cases are estimated to occur every year, with 300—400 TB deaths daily. To cope with this problem, the DOTS strategy has been implemented since 1998 and aims to achieve a case detection rate (CDR) of 70% and cure rate or success rate (SR) of 85% by the end of 2006. Figure 3 and 4 show the date on CDR and SR from 1997 to 2005.

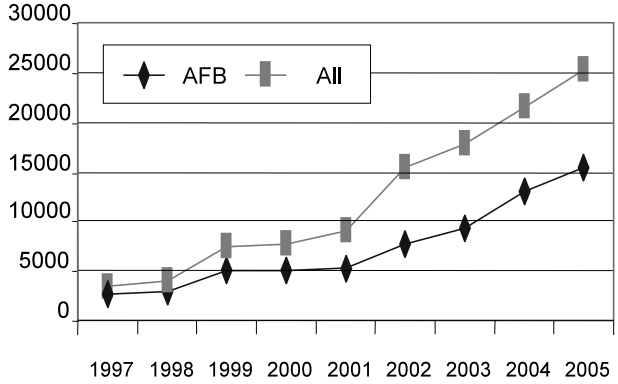


Figure 3. Number of Cases Detected 1987-2005 in the TBC Control Program

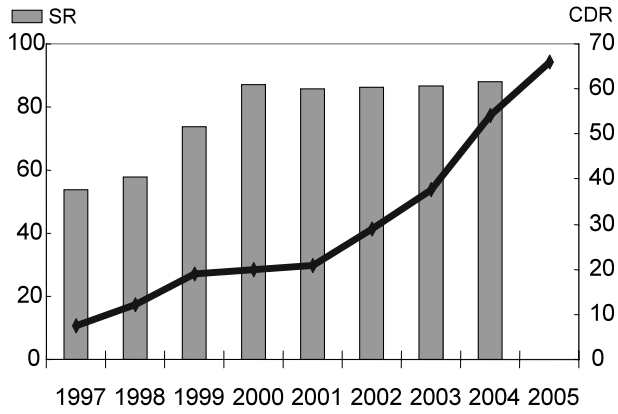


Figure 4. CDR and SR of AFB Positive Cases in Indonesia 1997-2005

TB is diagnosed mainly if acid-fast- bacilli (AFB) are found in the sputum, body fluids or tissue in combination with clinical symptoms. In special cases, chest radiograph and PPD skin test abnormalities are also taken into consideration The principal challenges in TB control include obtaining and continuing the political commitment of decision makers, and international funding for the support of TB control efforts. Multiple drug resistance is also a challenge that will have to be addressed in the near future.

MALARIA

Most malaria eradication programs in the past have been unsuccessful. Figure 5 shows the malaria endemicity in 2005 and figure 6 the malaria situation in Indonesia from 1989 to 2005.

Deforestation, mining, active rapid population migra-

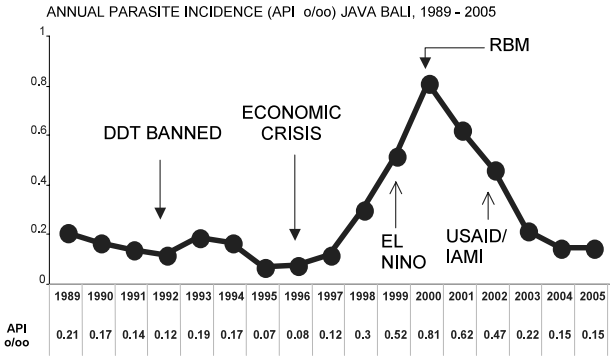


Figure 6. MALARIA SITUATION IN INDONESIA 1989-2005

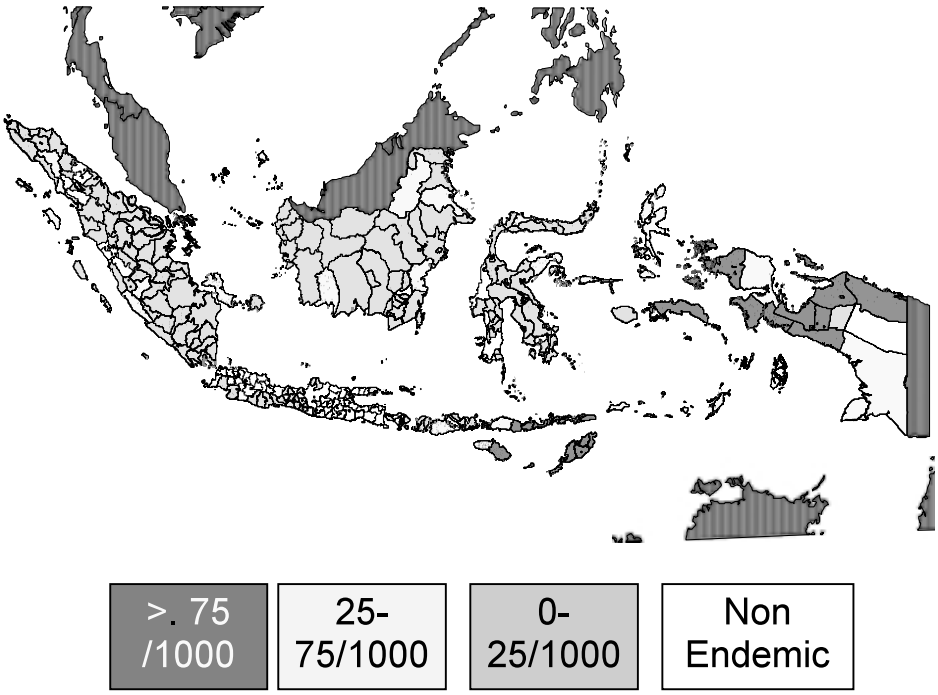


Figure 5. MALARIA ENDEMICITY DISTRIBUTION IN 2005

tion and other development activities have contributed to the resurgence of malaria in Indonesia. The roll back malaria strategy, which was implemented as GEBRAK Malaria in 2000, focuses on partnership. Malaria control strategy primarily focuses on vector and disease surveillance, early diagnosis and prompt treatment, integrated vector management and community participation.

The principal challenges to malaria control programs are effective coordination, long-term sustainability of vector control efforts, population migration and environmental changes. Anti-malaria drug resistance and insecticide resistance are also primary challenges for the future.

POLIO

Polio eradication has been targeted for the year 2008.

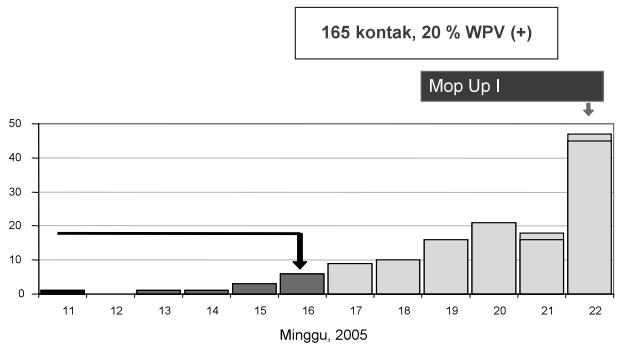


Figure 7. EPI TARGET DISEASES Polio epidemic in Indonesia, weeks 11-22

The polio eradication campaign is a good example of multi-partner leadership in that partners come from a broad array of organizations such as Rotary International, WHO UNICEF, CDC-Atlanta, USAID, AUSAID, and World Bank.

Poliomyelitis is caused by the poliovirus (P_1, P_2, P_3) transmitted via the orofecal route due to poor personal hygiene and environmental sanitation. Potent oral polio vaccine and injectable vaccine are available to combat the disease, and the former was included in the Routine Immunization Program in Indonesia in 1980. Universal Child Immunization coverage (≥80%) was achieved in 1990. National Immunization Days were designated in 1995, 1996 and 1997 consecutively as part of the global polio eradication campaigns launched in 1988 by WHO. A wild poliovirus-free status continued for 10 years from 1996, but an imported polio case was reported from Cidahu, Sukabumi in March 2005. This wild poliovirus was imported from Nigeria via Yemen and Saudi Arabia. Figure 7, 8, and 9 show the spread of wild poliovirus and efforts made to control the outbreak.

Since the outbreak in March 2005, there have been 305 lab confirmed WPV cases (303 in 2005 and 2 in 2006), and 46 VDPV cases. The most recent wild polio case was reported on 20 February 2006 from Aceh Tenggara district, NAD province

Since the outbreak in March 2005, the total number of lab confirmed polio cases was 305, including 303 in 2005

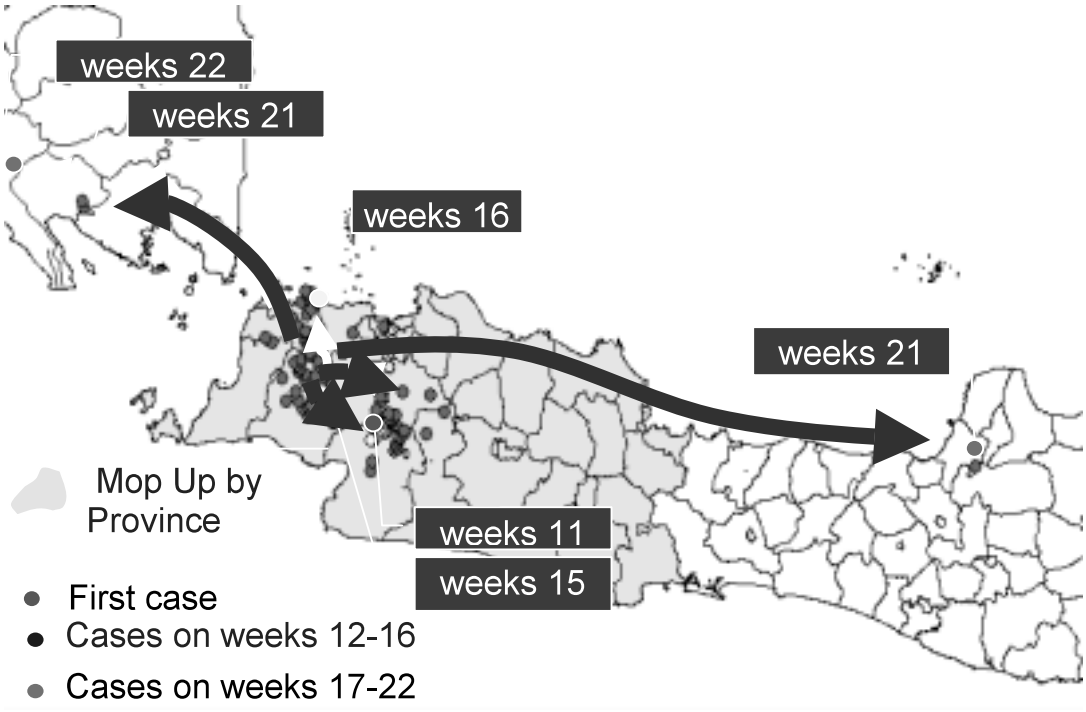


Figure 8. Wild Poliovirus Cases in Indonesia, March 2005- present

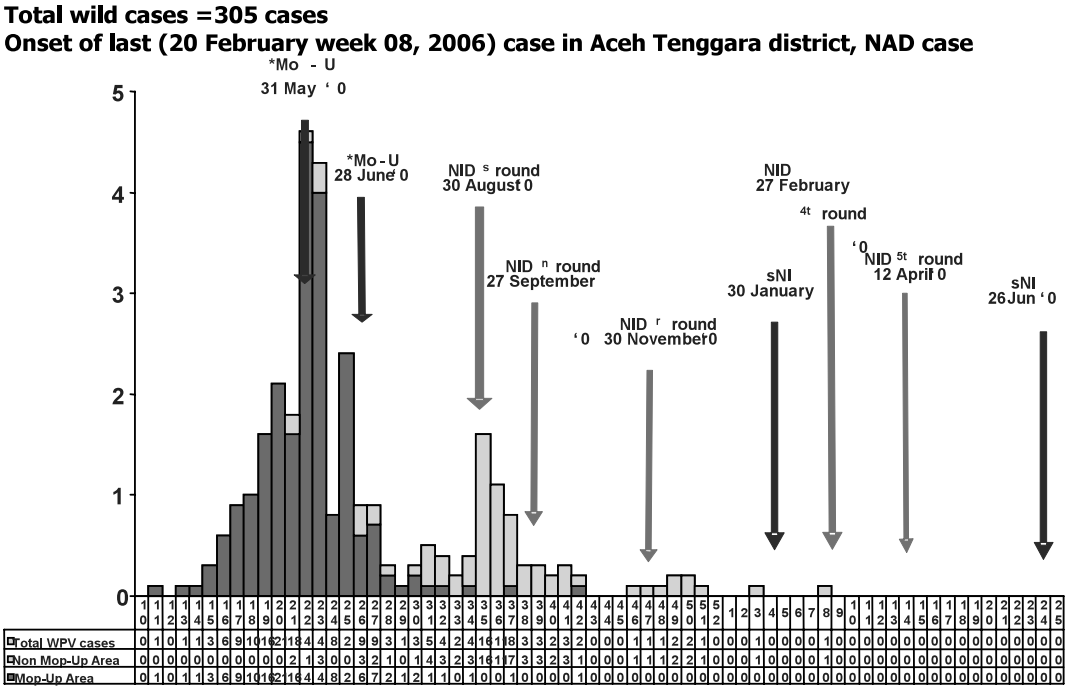


Figure 9. Wild Polio Cases by Week of Onset, Indonesia 2005-2006

and 2 cases in 2006, and 46 VDPV (vaccine derived polio virus) cases. After various efforts including mopping up, National Immunization Days (NID) and Sub NID, the last case was reported in 20 February 2006 from Southeast Aceh. A future challenge to EPI is the sustainability of the routine immunization, including both managerial and financial sustainability. A long-term plan is being developed to address future challenges.

HIV/AIDS

The Human Immunodeficiency Virus (HIV) causes acquired immunodeficiency syndrome (AIDS). The first HIV/AIDS case in Indonesia was reported in the late 80's from Bali, after which the disease spread rapidly to 32 out of the 33 provinces of Indonesia. The cumulative number of HIV

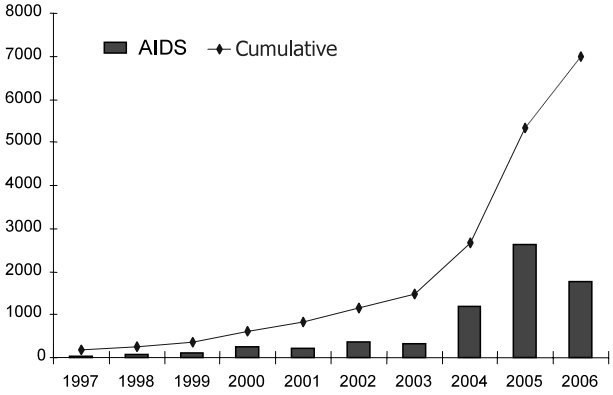


Figure 10. CUMULATIVE AIDS CASES UP TO THE END OF SEPTEMBER 2006

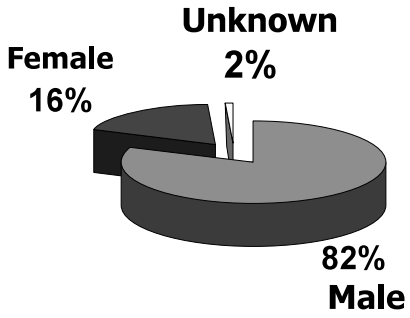


Figure 11. SEX DISTRIBUTION OF AIDS CASES UP TO THE END OF SEPTEMBER 2006

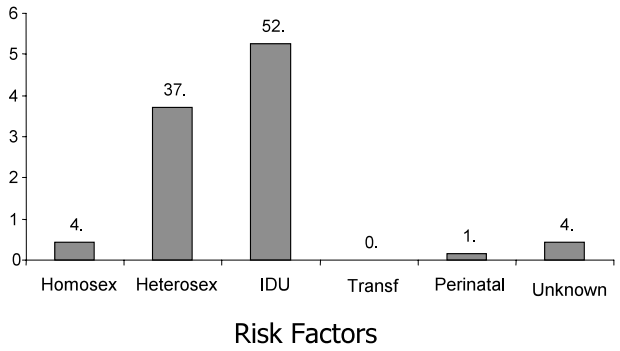


Figure 12. RISK FACTOR OF AIDS CASES BY MODE OF TRANSMISSION UP TO SEPTEMBER 2006

infection and AIDS cases reported up to September 2006 is 4617 and 6987, respectively. Figure 10 shows the trend of AIDS cases from 1997 to 2006 and figure 11 shows the sex distribution. The AIDS cases were predominantly male (82%), with females accounting for only 16% and unknown for 2%. Risk factors by mode of transmission are shown in figure 12. Intense and rapid spread of HIV was documented among injecting drug users (IDU) (52%), followed by heterosexuals (37.2%). The cumulative number of AIDS cases is expected to reach 93,968 to 130,000 by the year 2010. In Papua, pregnant women and newborn babies were infected by HIV. The main strategy to combat HIV infection includes efforts to prevent new infection, to promote comprehensive care, and to increase coverage of HIV infection through partnership. The principal challenges to HIV/AIDS control programs are intersector coordination, behavior changes, and human resources.

SARS (Severe Acute Respiratory Syndrome)

During the SARS outbreak in 2003, there were only 7 suspected and 2 probable cases admitted to hospitals in Indonesia. No confirmed case was reported. Strategies and actions taken during the SARS outbreak included the screening of incoming passengers from affected countries with thermo scanners, establishment and capacity building of 35 referral hospitals and 45 port health offices, public awareness and regional networking (ASEAN +3).

AVIAN INFLUENZA

Outbreaks of H5N1 infection in the poultry population were first reported in 2003. Human cases were first reported in July 2005. To date, 32 of 33 provinces of Indonesia have experienced H5N1 infection among the poultry population, while H5N1 infection in humans has been reported from only 9 provinces. Figure 13 shows the distribution of avian influenza outbreaks in animals and humans.

Strategies to control H5N1 transmission include the prevention of H5N1 transmission in the poultry population, surveillance, bio-security, case management, public awareness/risk communication, research and development. The main challenges to H5N1 control are coordination and resources.

NEGLECTED DISEASES

Leprosy, lymphatic filariasis, yaws, rabies, and Japanese encephalitis are considered to be neglected diseases. Very little attention has been paid to these diseases, and insufficient resources have been allocated for their control. Fortunately, international agencies and the international community are interested in leprosy and lymphatic filariasis and as a result we aim to eliminate leprosy and lymphatic filariasis by 2020. An integrated approach is being implemented to cope with the limited resources available.

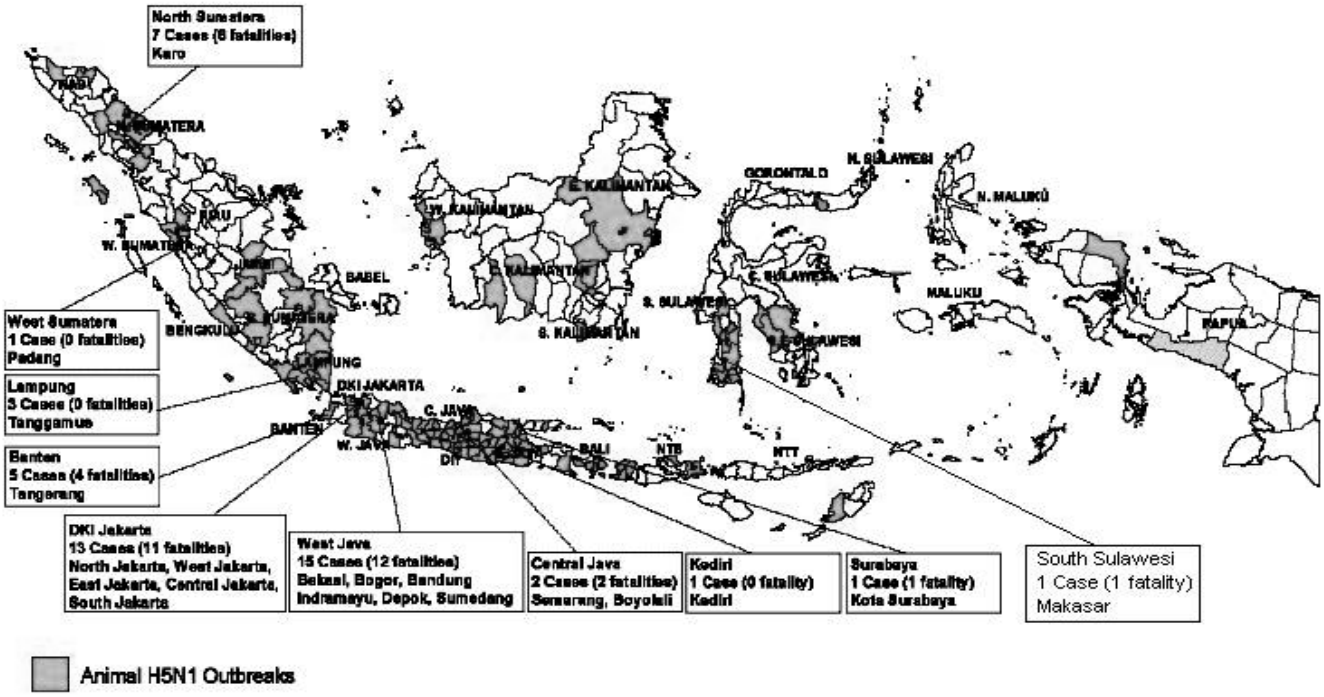


Figure 13. AVIAN INFLUENZA OUTBREAK IN ANIMALS AND HUMANS
H5N1 in animals: 30 provinces, 202 districts/munics. H5N1 in humans: 9 provinces, 24 districts/munics.

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ROLES OF MULTI-COUNTRY NETWORKING IN PREVENTION AND CONTROL OF EMERGING AND RE-EMERGING INFECTIONS

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Recieved 13, December, 2006

ABSTRACT: The emergence of new and the re-emergence of old infectious diseases reverses our previous belief that communicable diseases have been brought under human control. The development of antibiotic-resistant bacterial and fungal infections and the apparent lack of vaccines for many infectious diseases remind us of how vulnerable we are. Infections with SARS coronavirus, Nipah virus, and, more recently, H5N1 influenza virus in humans are just a few reality checks and more are expected. The problems of these emerging and re-emerging infections (ERI) give us several warnings. *First*, there are a number of pathogens, most of which are viral, that pose potential risks to human health and we know quite little about them. *Second*, many of these ERI are zoonotic. Effective control of zoonoses needs involvement and collaboration from the non-health sector. *Third*, since these ERI can easily spread across geopolitical boundaries, countries with a good public health infrastructure are not risk-free and should not be complacent. Surveillance and response efforts cannot be limited within one national boundary. *Fourth*, aside from the fundamental tools in disease control that we have, e.g. basic sanitation, personal hygiene, isolation and quarantine, and the newly-revised International Health Regulations, we have little other choices, e.g. drugs and vaccines. This limitation suggests that we could eventually be defenseless. *Fifth*, recent occurrences of certain ERI in some countries have demonstrated that damages caused in non-health terms, e.g. economic losses, can be significant. As a consequence, ERI may be dealt with as an economic problem while the human and health dimensions are ignored.

There are a number of guiding principles that we may have to adopt. *First*, no country can or is allowed to fight ERI alone, no matter how well-developed its economic condition and public health infrastructure. However, it should be noted that ERI is a national health security problem and that national sovereignty must be recognized when addressing the ERI issues. *Second*, ERI problems need a lot of non-health partners, e.g. business and agriculture. *Third*, we need to improve our capacity to do surveillance and to respond. Surveillance without response is pointless. Fourth, we need specific tools to help tackle ERI.

Networking is a mechanism through which countries can work together to fight ERI. A number of forums exist to address the problems of ERI, e.g. meetings of WHO and other UN agencies including FAO/OIE, ASEAN＋3, and ACMECS. In addition, several bilateral frameworks are in place to address ERI. However, it is important to note the particular nature of ERI and follow the above-mentioned guiding principles to avoid failure.

INTRODUCTION

Emerging and re-emerging infections, by definition, are new threats to human beings, although many of the pathogens causing the infections may have been with us for a long time without our due attention. In the course of human development, we are constantly challenged by threats, including those from infectious diseases. Discovery of effective antibiotics and the rapid progress of virology may instill a complacency that infectious diseases are under our complete control and we do not have to be wary of communicable diseases. However, the emergence of HIV/AIDS epidemic some twenty years ago and the recent outbreaks of SARS coronavirus, Nipah virus, and H5N1 influenza virus in humans reverse this belief. The threat is further aggravated by long-recognized-but-largely-ignored problems of antimicrobial resistance not only among bacteria but also viruses, fungi and even parasites. In other words, not only are we unable to control existing enemies, we are faced with more and stronger enemies. In addition, our most effective tools, e.g. antimicrobials, are becoming less effective.

There are several lessons to be leaned from our recent encounters with emerging and re-emerging infections (ERI).

First, the list of emerging and re-emerging infections is

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long and growing. If we visit the first issue of the journal "Emerging Infectious Diseases or EID" published in 1980, we can see the introductory paper by David Satcher of the US Centers for Disease Control and Prevention on ERI, as shown in Figure 1[1]. Although the paper is now over 20 years old, the list of EID in the paper was already long and, I am sure, if the list is revised, it will grow. Most of the diseases or syndromes on the list and the ones we have faced recently are quite strange to us. That means we know relatively little about them. Our relative ignorance of ERI is the major drawback in our fight against them.

Second, the origins of many recent ERI can be traced back to animal reservoirs or carriers or cases, e.g. civet cats in SARS, pigs in Nipah viral infection, and birds in H5N1 infection. Many ERI exist in animals long before they are transmitted to humans. Increased contact between human beings and animals increases the chance of infections jumping onto humans. Such includes raising domesticated or farm animals, contacting migratory animals such as birds, and hunting and eating exotic animals. Human medicine does not prepare doctors or public health professionals to handle diseases of animal origin. Therefore, there is a strong and urgent need for better and closer coordination between the veterinary sector and human sector.

Third, the readily transmissible nature of most ERI puts all countries at risk. SARS is a particularly contagious disease. Nipah encephalitis spreads rapidly among pigs and could potentially do so in humans if effective control in animals is not put in place rapidly. Migratory birds help spread H5N1 infections to all corners of the globe. The rate of the

Table 1. Major Etiologic Agents, Infectious Diseases Identified Since 1973*

Year	Agent	Disease	Reference
1973	Rotavirus	Major cause of infantile diarrhea worldwide	19
1975	Parvovirus B19	Fifth disease; Aplastic crisis in chronic hemolytic anemia	20
1976	*Cryptosporidium parvum*	Acute enterocolitis	21
1977	Ebola virus	Ebola hemorrhagic fever	22
1977	*Legionella pneumophila*	Legionnaires' disease	23
1977	Hantaan virus	Hemorrhagic fever with renal syndrome (HFRS)	24
1977	*Campylobacter* sp.	Enteric pathogens distributed globally	25
1980	Human T-cell lymphotropic virus-I (HTLV I)	T-cell lymphoma—leukemia	26
1981	*Staphylococcus* toxin	Toxic shock syndrome associated with tampon use	27
1982	*Escherichia coli* O157:H7	Hemorrhagic colitis; hemolytic uremic syndrome	28
1982	HTLV II	Hairy cell leukemia	29
1982	*Borrelia burgdorferi*	Lyme disease	30
1983	Human immunodeficiency virus (HIV)	Acquired immunodeficiency syndrome (AIDS)	31
1983	*Helicobacter pylori*	Gastric ulcers	32
1988	Human herpesvirus-6 (HHV-6)	Roseola subitum	33
1989	*Ehrlichia chaffeensis*	Human ehrlichiosis	34
1989	Hepatitis C	Parenterally transmitted non-A, non-B hepatitis	35
1991	Guanarito virus	Venezuelan hemorrhagic fever	36
1992	*Vibrio cholerae* O139	New strain associated with epidemic cholera	37
1992	*Bartonella* (= *Rochalimaea*) *henselae*	Cat-scratch disease; bacillary angiomatosis	38, 39
1993	Hantavirus isolates	Hantavirus pulmonary syndrome	40
1994	Sabiá virus	Brazilian hemorrhagic fever	41

* Compiled by CDC staff. Dates of discovery are assigned on the basis of the year the isolation or identification of etiologic agents was reported.

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Figure 1: What emerging and re-emerging infections (ERI) to expect?

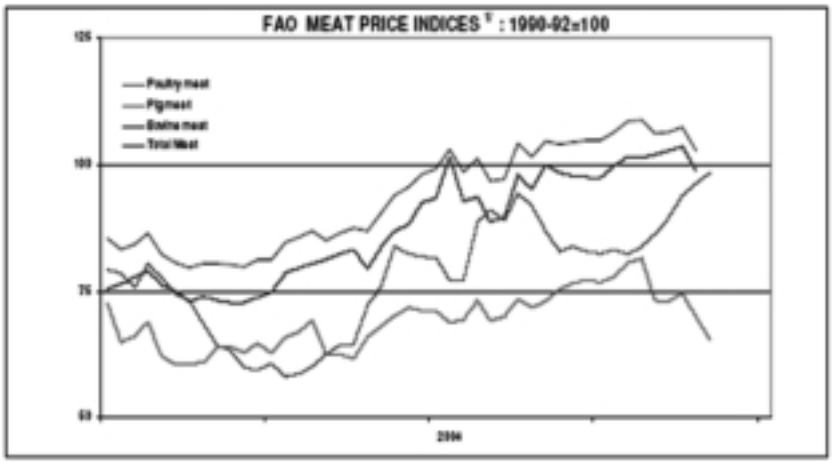

Figure 2: Global meat prices as a consequence of HPAI outbreaks (Source: FAO)

spread is virtually independent of geopolitical boundaries and economic, social and public health development status, although countries with better-developed public health systems and infrastructures may be able to control the diseases more effectively and efficiently. This is a reminder that ERI cannot be fought in one country alone.

Fourth, most ERI not only take lives but also result in reduced or lost livelihoods of human beings. SARS reduced global air travel. Nipah viral infection resulted in the culling of a large number of pig reservoirs and cases. H5N1 diseases among poultry and in humans resulted in the massive culling of domesticated chickens and ducks. There is evidence that the livelihood of agricultural workers has been affected and the price of chicken meat (as the direct impact) and of other meats such as pork and beef (as a compensatory mechanism) increased significantly as a result of the culling, as shown in Figure 2. Since direct and indirect economic losses from ERIoutbreaks are enormous, many people and organizations are concerned that economic issues may eclipse problems related to the health of the people.

Fifth, we do not have specific interventions for most ERI. Most ERI are viral, and there are no specific antivirals nor vaccines for the infections. Although efforts are underway to develop a vaccine for SARS, it is not yet available. We have specific vaccines for circulating strains of human influenza viruses, e.g. type A (H1, H3), type B, but specific vaccines need to be developed annually to match circulating strains. Furthermore, although we believe that vaccines produced ion the same principle as seasonal influenza vaccines will be effective against H5N1 and other pandemic influenza strains, we are yet to come to terms with how to make an adequate amount of effective vaccines for a pandemic.

Mechanisms available to combat ERI

Given the above characteristics of ERI, it is almost impossible to discuss the prevention and control of ERI in an isolated single-country context. Most prevention and control efforts involve other countries. International collaboration is a much-welcomed term in current public health practices. Multi-national mechanisms are currently available at the global level, e.g. World Health Organization (WHO; http://www.who.int/csr/en/), World Organization for Animal Health (OIE; http://www.oie.int/eng/en_index.htm), and Food and Agriculture Organization (FAO; http://www.fao.org/ag/againfo/subjects/en/health/diseases_cards/special_avian.html). A number of regional economic forums also address the issue of ERI, e.g. Asia-Pacific Economic Cooperation (APEC; http://www.apec.org), Association of Southeast Asian Nations (ASEAN; http://www.aseansec.org), and Ayeyawady-Chaophraya-Mekong Economic Cooperation Strategy (ACMECS;: http://www.geis.fhp.osd.mil/aboutGEIS.asp). In addition, there are a number of bilateral collaborations that specifically address ERI-related issues, e.g. those organized by the US Centers for Disease Control and Prevention and the US Department of Defense.

Guiding principles for dealing with ERI

The operation of international collaborations on ERI requires a few common principles. The following principles are proposed as overarching principles that could guide international collaboration, especially in fighting ERI.

First, no country can or is allowed to fight ERI alone, no matter how well-developed its economic condition or public health infrastructure. The rapid transmissibility across geopolitical boundaries demands that we all work together. No country is immune and no one can be complacent. It is therefore a wise strategy for wealthy countries, e. g. countries in North America and Western Europe, to come

Figure 3: International (global and regional) forums for emerging and re-emerging infections

to Asia to fight H5N1 at the forefront. However, care should be taken to avoid infringement on national sovereignty. A delicate balance has to be maintained.

Second, ERI problems call for a lot of non-health partners, e.g. business and agriculture, and effective control is not possible without involvement of these partners. It is encouraging to see, at the global revel, a close working relationship between human health and animal health sectors in fighting H5N1 diseases. However, this working relationship may not be seen at the country and sub-country levels. Lack of such close collaboration at all levels could easily fail us. Therefore, mechanism(s) must develop to forge and foster such collaboration in the fight against ERI.

Third, we need to improve our capacity to conduct surveillance and to respond. The importance of surveillance cannot be stressed enough. However, we should not forget that the definition of surveillance is “information for action”. This means that surveillance without response is pointless and may be useless. At the same time, response without surveillance is blind and can be really harmful.

To respond to ERI more effectively, we need specific tools to tackle ERI. Although basic health sanitation, e.g. hand wash, cough etiquette, and other improvements in general hygiene, is effective in reducing human-to-human transmission of ERI, we need more effective interventions. Development of vaccines and new drugs are tedious and time-and resource-consuming, but we need to invest in them. If “we”, the private vaccine and drug manufacturers, are not ready, then “we”, the governments, need to kick in. The role that governments can play ranges from providing sufficient supplies of drugs and vaccines (from available channels) to support for research, development and production of drugs and vaccines. Unfortunately, most governments do not possess the capacity nor carry the leverage to enhance research, development and production of the capacity necessary. Most of the capacity to produce drugs and vaccines belongs to multi-national private companies. In order for a country at risk of ERI epidemics to be self-sufficient in the vaccines and drugs needed to combat ERI, the government may need to promote technology transfer from private vaccine-and drug-manufacturing companies. This technology transfer may require the cooperation of international organizations, such as the World Health Organization.

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EXPLORING FRESH COLLABORATIVE INITIATIVES FOR COMBATING INFECTIOUS DISEASES IN THE PHILIPPINES

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Eight of the 10 leading causes of morbidity in the Philippines are infectious in nature, including pneumonia, diarrhea, bronchitis, influenza, tuberculosis, malaria, chicken pox and measles. Pneumonia and tuberculosis continue to cause a significant number of deaths across the country and persist to be among the 10 leading causes of mortality. The diminishing burden of communicable diseases as major causes of death may be attributed to improved health technology and health care delivery systems. Appropriate strategies and technologies include immunization, improved sanitation and personal hygiene, better nutrition, early treatment and steady supply of antibiotics made available at the community or at first level health facilities. However, the persistence of communicable diseases as major causes of morbidity points to the fact that much still needs to be done to reduce the occurrence of illness through preventive and promotive health measures.

A 20-year (1981-2000) trend analysis reveals a general decline in number of cases, and number of deaths from communicable diseases of public health importance, which include, among others, tuberculosis, malaria, schistosomiasis, tetanus, diphtheria, pertussis, measles, rabies, diarrheal diseases and pneumonia.

Although tuberculosis remains to be a major public health concern in recent years, effective case finding, disease management with DOTS strategy, and partnership with the private sector have led to significant improvements in the prevention and control of the disease.

Successful nationwide immunization campaigns undertaken over the years have resulted in the eradication of poliomyelitis and a continuous decline in the incidence of measles, tetanus, diphtheria and pertussis. I mproved sanitation, better nutrition and increased awareness of the community and appropriate management with oral rehydration resulted in the reduction of deaths from diarrhea. Reduction of mortality from pneumonia, especially among children, may be attributed to early recognition of the public and early diagnosis and treatment with appropriate antibiotics.

HIV/AIDS is “slow and low” in the Philippines, which means that transmission is slow and prevalence is low. However, it threatens to grow into a major epidemic in the country.

Although malaria is no longer a leading cause of death, it has remained among the leading causes of morbidity in the country, especially in rural areas. The fatal consequences and cyclical occurrence of outbreaks of dengue every three to five years is a public health concern. Efforts to eliminate filariasis are hindered by limited resources for annual mass treatment in endemic areas. Although the prevalence is declining, it is a source of concern that for some endemic infectious diseases such as schistosomiasis, areas previously identified as non-endemic areas have been found to have new cases.

Emerging infectious diseases, i.e. newly identified or previously unknown infections including re-emerging IDs, cause serious public health problems if not contained as close as possible to their source. The inherent unpredictability of a variety of previously known infections and unknown diseases can limit the responsiveness of even the most organized health system.

In 2003, the sudden and unexpected emergence of SARS presented an opportunity to initiate measures to strengthen local and international surveillance for emerging and re-emerging infections as well as to strengthen quarantine and isolation measures, build capacity in laboratory di-

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agnosis and clinical management, strengthen structure, systems and procedures for triaging, infection control, surveillance and epidemiologic investigation, hospital referral, advocacy and risk communication.

Avian influenza (AI) or bird flu due to the highly pathogenic influenza virus, H5N1, is a grave threat to humanity. The unpredictability of the influenza virus and the serious possible consequences of a pandemic warrant constant vigilance and good planning in order to reduce the impact of a pandemic.

The unexpected and unusual increase in meningococcal disease, with meningococcemia as a predominant form, in the Cordillera Autonomous Region led to the death of at least 50% of cases in the early stage of this occurrence, causing public anxiety and seriously affecting the economy, particularly in Baguio City. There is a need to develop the regional capability for laboratory diagnosis and to conduct research studies on population carriage and serogroups predominant in the Philippines.

Hepatitis C is an emerging infection in the Philippines with high prevalence among IV drug users studied. There is a need for more epidemiological data among contacts of positive cases to determine the extent of transmission and to monitor and avert the progression of the disease.

Leptospirosis increases during the rainy season as the aftermath of flooding but cases are also recognized in regions with vast farmlands. There is a need to establish a laboratory-based surveillance for leptospirosis, to determine the burden of disease in the Philippines, and to identify the factors for susceptibility and severity and the circulating serovars causing various degrees of severity of disease.

Tuberculosis has been identified as a leading cause of CNS infections. Other etiologies: varicella, herpes zoster, Japanese encephalitis. There is a need for a continuing laboratory-based surveillance of meningitis and encephalitis as identification of a specific etiology will serve as a basis for specific treatment and vaccination programs.

The current data on Japanese encephalitis may be just the tip of an iceberg. The national and regional incidence and burden of disease of Japanese encephalitis and the regional distribution among its animal hosts need to be determined as a basis for national policy and programs.

Despite the continuing decline in mortality from pneumonia, more efforts can be done to strengthen measures for its prevention and control. There is a need to strengthen laboratory capabilities to conduct etiologic identification and antimicrobial resistance surveillance for appropriate management of cases. Infection control in the health care setting also needs to be strengthened.

The following areas for possible technical cooperation may be considered: strengthening systems for early recognition of disease and response of local units, establishment of real-time surveillance and information systems, building diagnostic and management capacities for infectious diseases in designated national and sub-national facilities, epidemiological studies on emerging infectious diseases such as meningococcal disease, melioidosis, Legionella, Hepatitis C, etiologies of CNS, diarrhea and pneumonia, burden of disease as basis for public health programs for influenza, JE, leptospirosis, developing well-trained and well-equipped response teams from the national to the local level, preparedness assessment through simulation and other similar exercises, operational studies: evaluation of preparedness of LGUs, of hospitals, public awareness on various EIDs.

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A NEW SPECIES OF *SIMULIUM* (*NEVERMANNIA*) FROM THE OGASAWARA (BONIN) ISLANDS, JAPAN (DIPTERA: SIMULIIDAE)

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ABSTRACT: *Simulium* (*Nevermannia*) *satakei* sp. nov. is described on the basis of the pupa and mature larvae collected from the Ogasawara (Bonin) Islands in Japan. This new species, tentatively (due to lack of the adult stage) assigned to the *vernum* species-group of the subgenus *Nevermannia*, is characterized in the pupa by four gill filaments lacking transverse ridges, and in the larva by a small, M-shaped postgenal cleft, antenna without hyaline bands, and simple rectal papilla. The morphological differences among this new species and the two known species, *S.* (*N.*) *uemotoi* from Japan and *S.* (*N.*) *karzhantacum* from Uzbekistan and Turkmenistan, are noted. This is the second species of the family Simuliidae from the Islands.
Key words: *Nevermannia*, Simuliidae, *Simulium*, black fly, Ogasawara Islands, new species

Until now, *Simulium* (*Nevermannia*) *bonninense* (Shiraki), a member of the *vernum* species-group of the subgenus *Nevermannia* Enderlein, was the only species of the family Simuliidae so far recorded from the Ogasawara (Bonin) Islands located in the Pacific Ocean, ca. 1,000 km south-southeast of Tokyo (Shiraki, 1935; Stone, 1964; Saito et al., 1974; Takaoka et al., 1999). Recently, a pupa and a few larvae of an unknown species were collected together with some pupae and larvae of *S.* (*N.*) *bonninense* from a small stream in Hahajima, one of the Ogasawara Islands.

This is described here as a new species and is tentatively (due to lack of the adult stage) assigned to the *vernum* species-group within the subgenus *Nevermannia*.

The terms for morphological features used here follow those of Takaoka (2003). Holotype and paratype specimens of the new species are deposited at the Department of Infectious Disease Control, Faculty of Medicine, Oita University, Oita, Japan.

Simulium (Nevermannia) satakei sp. nov.

DESCRIPTION. **Pupa**. Body length 2.0 mm. ***Head***. Integument (Fig. 1A) yellowish, moderately covered with small tubercles; antennal sheath (Fig. 1B) sparsely covered with small tubercles; frons with 2 medium-long slender trichomes (Fig. 1C) on each side; face with 1 long stout trichome (Fig. 1D) on each side, which is 1.3−1.7 times as long as those of frons. ***Thorax***. Integument yellowish, moderately covered with small tubercles, and on each side with 3 long stout simple trichomes (Fig. 1E) mediodorsally, 2 long simple trichomes (1 somewhat shorter and more slender than the other) (Fig. 1F) anterolaterally, 1 medium-long somewhat stout simple trichome (Fig. 1G) posterolaterally, and 3 short slender simple trichomes (Fig. 1H) [though 1 additional medium-long slender trichome (Fig. 1I) was present on the right side] ventrolaterally. Gill (Fig. 1J) with 4 slender thread-like filaments arranged in dorsal and ventral pairs arising from short common basal stalk; stalk of ventral pair slightly shorter than common basal stalk but slightly longer than the stalk of dorsal pair; dorsalmost filament and ventralmost one basally diverged vertically at a right angle when viewed laterally; all filaments subequal in thickness to one another; lengths of all filaments not measurable due to loss of apical portion except dorsal filament of dorsal pair (2.7 mm long) and ventral filament of ventral pair (2.1 mm long) of right gill; all filaments light yellow, gradually tapered toward apex, furnished with annular furrows but lacking ridges (Fig. 1K), and densely covered with minute tubercles on outer surface. ***Abdomen***. Dorsally, all segments weakly sclerotized and pale yellow; segments 1 and 2 sparsely or moderately covered with small tubercles (Fig. 1 L); segment 1 with 1 medium-long simple slender seta (Fig. 1M) on each side; segment 2 with 1 medium-long simple slender seta and 5 short dark spinous setae (Fig. 1N) on each side; segments 3 and 4, each with 4 dark stout hooks and 1 short spinous seta on each side; segments 5−9 each with spine-combs and comb-like groups of minute spines lying transversely along anterior margin (Fig. 1O) on each

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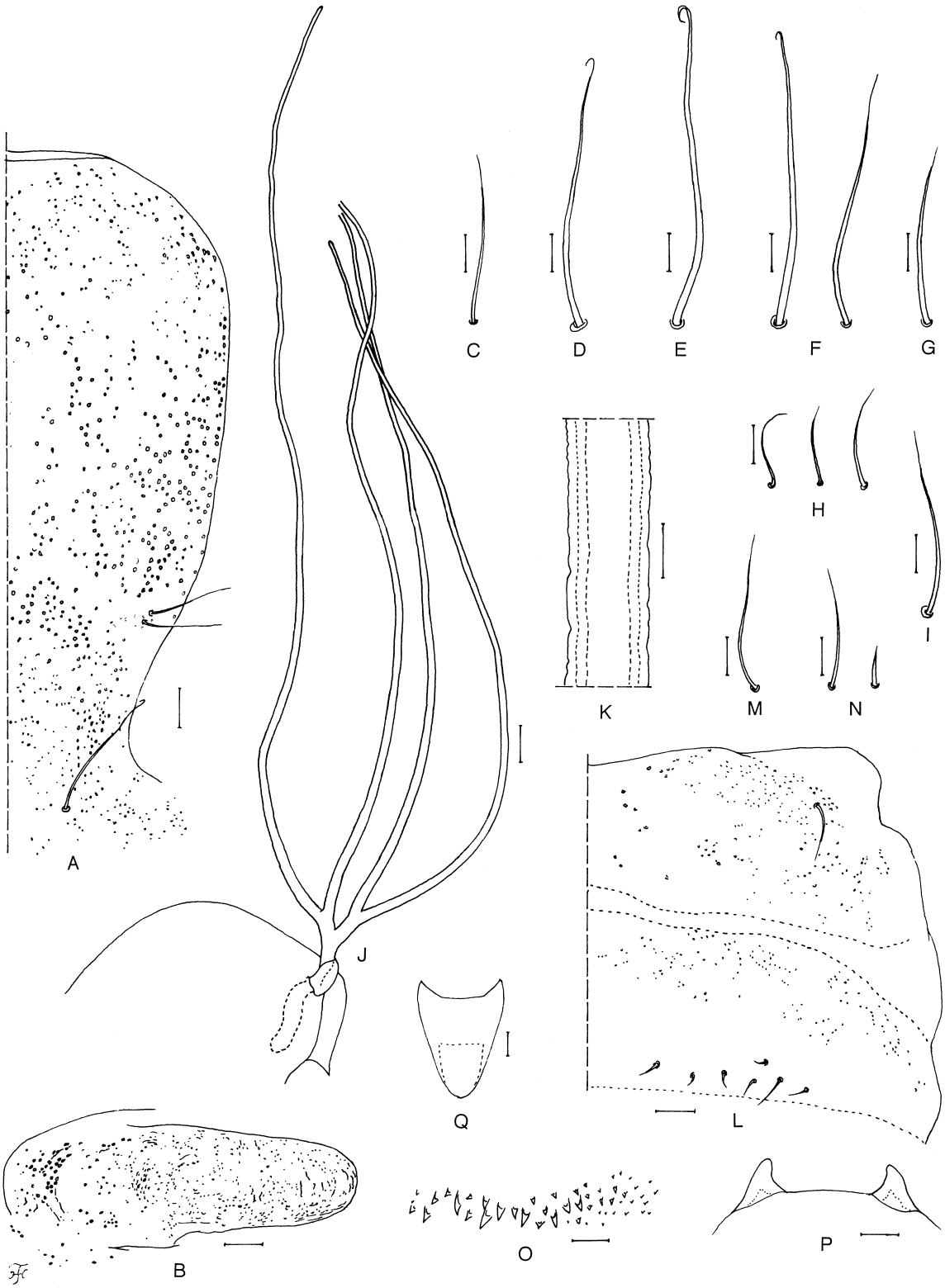

Fig.1. Pupa of *Simulium* (*Nevermannia*) *satakei* sp. nov.
A, frons and part of face (left half); B, antennal sheath; C, frontal trichome; D, facial trichome; E−I, thoracic trichomes (E, mediodorsal; F, anterolateral; G, posterolateral; H and I, ventrolateral); J, gill filaments (right side; lateral view); K, basal part of gill filament showing lack of transverse ridge; L, dorsal surface of abdominal segments 1 and 2 (right half); M, medium-long simple slender seta on dorsal surface of 1st abdominal segment; N, medium-long simple slender seta and short spinous seta on dorsal surface of abdominal segment 2; O, spine-combs and comb-like groups of minute spines on abdominal segment 9 (right half); P, terminal hooks (end view); Q, cocoon (dorsal view). Scales. 0.5 mm for Q; 0.1 mm for J; 0.04 mm for A, B and L; 0.02 mm for C−I, K, M, N, O and P.

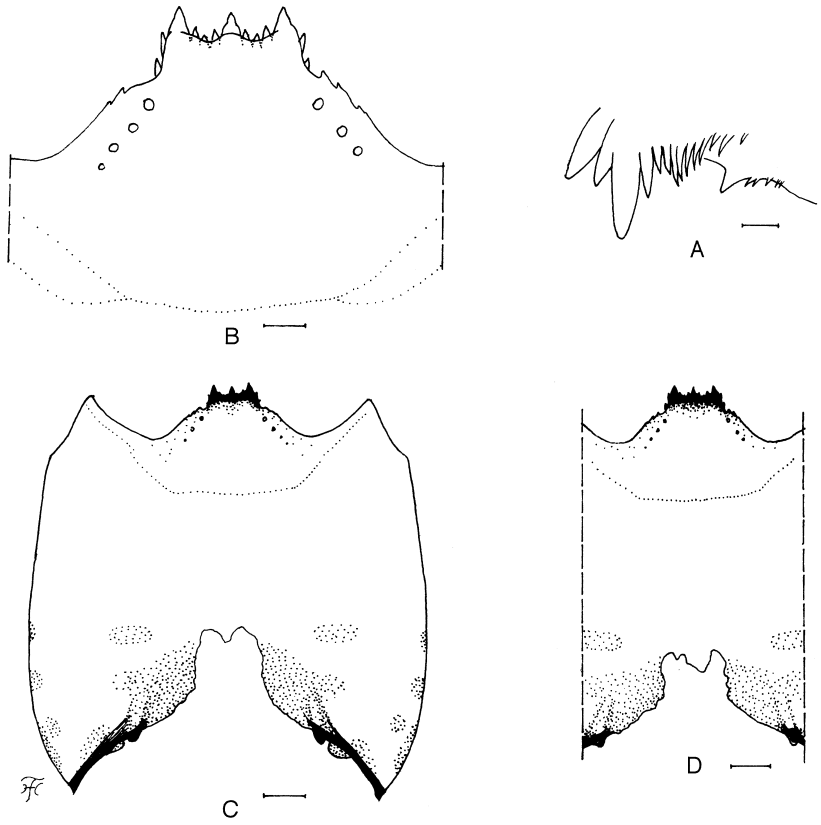

Fig.2. Mature larva of *Simulium* (*Nevermannia*) *satakei* sp. nov.
A, mandible; B, hypostoma; C and D, ventral surfaces of head capsules showing postgenal clefts of different sizes and shapes. Scales. 0.05 mm for C and D; 0.02 mm for B; 0.01 mm for A.

side; segment 9 with pair of cone-shaped terminal hooks (Fig. 1P). Ventrally, segments 3−8 nearly transparent and segment 9 weakly sclerotized and pale yellow; segment 3 with 3 short simple setae on each side; segment 4 with 1 simple dark hooklet (slightly shorter and smaller than those on segments 5−7) and 3 short simple setae on each side; segment 5 with 2 bifid dark hooks and a few short simple setae on each side; segments 6 and 7 each with 1 bifid dark inner hook and 1 simple or bifid dark outer hook, and a few short simple setae on each side; segments 4−8 with comb-like groups of minute spines. Segment 9 with short simple seta on each lateral side. ***Cocoon*** (Fig. 1Q). Simple, wall-pocket-shaped, moderately woven, with anterior margin somewhat thickly woven, and extending ventrolaterally; floor woven on posterior 2/5; individual threads visible; 2.5 mm long by 2.0 mm wide.

Mature larva. Body length 3.5−4.0 mm. Body color creamy yellow. Cephalic apotome yellow; head spots all positive and medium brown. Lateral surface of head capsule yellowish except eye-spot region yellowish-white, with eyebrow light brown; 2 large and 1 small spots just before posterior margin, as well as 2 small spots below eye-spot region all positive and medium brown. Ventral surface of head capsule yellow; 1 elongate spot on each side of postgenal cleft positive and medium brown. Cervical sclerites composed of 2 rod-like small pieces, not fused to occiput, widely separated from each other. Antenna composed of 3 segments and apical sensillum, much longer than stem of labral fan; proportional lengths of 1st, 2nd, and 3rd segments 1.00: 0.62−0.78: 0.83−0.93. Labral fan with about 34 rays. Mandible (Fig. 2A) with 1st comb-tooth longest; 2nd and 3rd comb-teeth subequal in length to each other; mandibular serrations composed of 2 teeth (1 large and 1 small); large tooth at a right angle to mandible on apical side; 2−4 supernumerary serrations present. Hypostoma (Fig. 2B) with a row of 9 apical teeth, the median tooth and corner teeth being most prominent, and median tooth of 3 intermediate teeth on each side smallest; lateral margins with well developed teeth; 3 or 4 hypostomal bristles in a row, subparallel to, or slightly diverging from, lateral margin on each side. Postgenal cleft (Fig. 2C,D) small, M-shaped, 0.41−0.64 times as long as postgenal bridge. Thoracic and abdominal cuticle almost bare except dorsal surface of a few posterior segments sparsely to moderately covered with col-

orless minute setae and areas on both sides of anal sclerite moderately covered with colorless short setae. Rectal scales absent. Rectal papilla simple, without secondary lobules. Anal sclerite X-shaped, anterior arms 0.8 times as long as posterior ones; accessory sclerite absent. Ventral papillae present ventrolaterally. Posterior circlet of hooks with about 64 rows of up to 15 hooks per row.

Female and **Male**. Unknown.

TYPE SPECIMENS. Holotype pupa with its associated cocoon, collected from a small shaded stream (width about 10 cm) slowly flowing in a forest, located on the right side of Chibusa Dam, Hahajima, Ogasawara Islands, Tokyo, Japan, 18.VI.2005, by K. Satake. Paratypes: 2 mature larvae and 1 immature larva, same locality and data as those of the holotype.

ETYMOLOGY. The species name *satakei* honors Dr. K. Satake, who collected this new species.

REMARKS. This new species is tentatively assigned to the *vernum* species-group of the subgenus *Nevermannia* by having the four gill filaments per side in the pupal stage, the antennae without any transverse hyaline bands, the mandible with supernumerary serrations, the main tooth of the mandibular serrations at a right angle on the apical side to the mandible, the hypostoma with serrated lateral margins, and the ventral papillae well developed in the larval stage.

The larva of this new species is very similar to that of *S*. (*N*.) *uemotoi* of the *vernum* species-group from Japan (Sato et al., 2004): it shares several characteristics including the small, M-shaped postgenal cleft and the simple rectal papilla. There are some differences, however, in the relative length of the three segments of the larval antennae (1.00: 0.62−0.78: 0.83−0.93 versus 1.0: 1.2−1.4: 0.8−1.0) between the two species. On the other hand, the pupa of this new species is easily distinguished from that of *S*. (*N*.) *uemotoi* by the following characteristics (those of *S*. (*N*.) *uemotoi* are shown in parentheses): frontal trichomes in two pairs (in three pairs), antennal sheath sparsely covered with small tubercles (bare), transverse ridges on the gill filaments absent (present), dorsal surface of the abdominal segments 1 and 2 sparsely covered with minute tubercles (densely and neatly covered with minute tubercles), and spine-combs on the abdominal segment 9 distinct (absent or indistinct if any).

This new species is also similar to *S*. (*N*.) *karzhantacum* (Rubtsov, 1956) from Uzbekistan and Turkmenistan, which has a similarly shaped larval postgenal cleft, but differs in the pupal stage from the latter species by lacking the transverse ridges on the surface of the gill filaments.

Simulium (*Nevermannia*) *satakei* sp. nov. represents the second species recorded from the Ogasawara Islands. It should be noted that this new species is not closely related to *S*. (*N*.) *bonninense*, which to date had been the only species prevalent in the islands, because there are distinct differences in the relative length of the first and second segments of the larval antennae and in the shape of the larval postgenal cleft as well as in the presence or absence of spine-combs on the dorsal surface of the fifth and ninth segments of the pupal abdomen between the two species.

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UVULECTOMY AND OTHER TRADITIONAL HEALING PRACTICES: TRADITIONAL HEALERS' PERCEPTIONS AND PRACTICES IN A CONGOLESE REFUGEE CAMP IN TANZANIA

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ABSTRACT: Little is studied about traditional healers' perceptions toward and practice of uvulectomy, which is known as a traditional surgical practice mainly in Africa and which sometimes results in severe complications. This study aimed to clarify the perceptions toward and practice of uvulectomy and the other traditional healing practices of traditional healers in a Congolese refugee camp in Tanzania. Interviews were conducted with 149 traditional healers, comprised of 59 registered, 68 non-registered and 22 faith healers.

A total of 1.7% of the registered healers and 8.8% of the non-registered healers had ever conducted uvulectomy on children (a median of 2 months to a median of 3 years of age) and had received cash or domestic fowls equivalent to US$1-3 per operation. Although over 80% of the respondents believed traditional treatments to be more effective than modern medicine, less than 20% considered uvulectomy beneficial and in fact about 40% considered it to be harmful. The respondents raised cough, vomiting, appetite loss and other symptoms as an indication for uvulectomy, and death, bleeding, throat pain and other symptoms as harmful effects associated with uvulectomy. In this camp, the healers also performed other surgical procedures, such as male and female circumcision, tattoos and scarification.

In conclusion, only a limited number of the traditional healers believed that uvulectomy is beneficial and performed it on infants and young children, and these were mainly non-registered healers who had relatively little collaboration with modern health professionals. In refugee settings where modern health professionals might not be familiar with traditional healing, it is considered crucial to assess the risks of ongoing traditional practices and to strive to achieve more strategic communication between modern and traditional health providers.
Keywords: traditional healing, healers, uvulectomy, perception, Congolese refugees, Tanzania

INTRODUCTION

The persisting conflicts in the Great Lakes region of Africa have caused the flow of a large number of refugees into the United Republic of Tanzania. Tanzania has maintained an open-door policy since its independence, and, as a result, it hosted approximately 520,000 refugees including more than 370,000 from Burundi and about 140,000 from Congo-Kinshasa, 3,500 from Somalia, and 2,700 from Rwanda by the end of 2002 [1].

In response to the extremely poor health status of these refugees, modern health care services have been provided intensively in refugee camps, when possible, by skilled or trained refugees and persons from the host community and aid organizations. However, there are still many refugee communities where traditional healing practices are strongly preferred.

While modern health intervention has contributed greatly to the improvement of the heath status in many camps, efforts to reduce infant mortality have stagnated in some camps [2]. Although no survey was conducted there, modern health providers in these camps suspected a traditional surgical practice called 'uvulectomy' to be one of the causes. Uvulectomy is a procedure in which the uvula is severed. It is a traditional healing practice used mainly in Africa, sometimes leading to serious complications [3, 4, 5]. However, no study has shed light on the perceptions of traditional healers as to its beneficial and adverse effects or its actual practice, especially in refugee settings. In the refugee camps, moreover, little was known about perceptions toward or practices of traditional healing methods other than uvulectomy.

This study aimed to determine the traditional healers' perceptions and their implementation of traditional healing practices, with special reference to uvulectomy.

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PARTICIPANTS AND METHODS

Study area

We selected Lugufu Camp, one of the biggest refugee camps in Kigoma Province, western Tanzania. It accommodated about 50,000 refugees from Congo-Kinshasa at the time of our study, and it continued to grow by an average of 1,000 refugees per month due to persistent armed conflicts, political instability, and deteriorating humanitarian conditions in that country. The camp was in the post-emergency phase with a crude mortality rate of 0.65 deaths/10,000 persons/day and an under-five mortality rate of 1.88/10,000 persons/day in 2000, thus indicating that the relief programs had successfully kept the health situation under control. [6]

Participants

Since there was no reliable document or registration method to identify traditional healers in the study site, we gathered information through preliminary interviews with modern health professionals and community health workers in the camp prior to the study. The results indicated that there were three types of healers, namely, registered traditional healers, non-registered traditional healers and faith healers. In the local language, the three types of healer are called 'MFUMU' or 'MTEE', 'MLAKO', and 'BAYUMBE' or 'MAHA WA ASA'O', respectively The 'MFUMU' or 'MTEE' was registered with the Ministry of Health in Congo-Kinshasa and was certified to examine and treat patients, supposedly with herbs and other medicinal subjects. The 'MLAKO' provided traditional healing practices without certification. The 'BAYUMBE' or 'MAHA WA ASA'O' was a faith healer, also referred to as a 'prayer leader', 'elder prayer', 'father/mother of prayer' or 'a director of prayer', who usually organized religious gatherings and provided healing mainly through prayers.

Study Preparation

Permission to carry out the study was obtained from the UNHCR and Tanzania Red Cross Society, with the support from the International Federation of Red Cross and Red Crescent Societies, organizations that took responsibility for health and other related activities in the camp. We made a list of traditional healers that had been identified in the camp by Congolese community health workers, and invited all of them to participate in our study. Informed consent was obtained, and finally all the healers in the list, 149 individuals in total, agreed to participate.

Eighteen Congolese refugees engaged in community health services speaking and writing English and Swahili were recruited and trained as interviewers for the study. We also recruited and trained Tanzanian health personnel competent in speaking and writing both English and Swahili as study supervisors. The authors were responsible for the training of both interviewers and supervisors and checked and confirmed the quality of supervisions. The study was conducted from May to July 2001.

Questionnaire

Semi-structured interviews were designed to explore traditional healers' perceptions toward and use of uvulectomy and other traditional healing practices, and their attitude toward modern health care services. Background information gathered from each respondent included religion, years of education, and the person from whom the healing practices were learned. Special efforts were made to obtain, in the respondent's own words, of the healing practices and their indications, and the perceived benefits and harm of uvulectomy. The respondents who used herbal medicine were further asked about the type of herbal medicine, their preparation, usage and indications.

To quantify the perceived benefits and harm of traditional practices, the interview also included the following questions: "In comparison to modern medicine, how effective do you think your traditional treatments are?" with four choices "All of my treatments are more effective than modern medicine", "Some of my treatments are more effective than modern medicine", "None of my treatments are more effective than modern medicine" and "I don't know"; "To what extent do you trust modern doctors?" with four choices "very much", "somewhat", "not at all", and "I don't know."; "To what extent do you think uvulectomy is beneficial or effective?" with four choices "very much", "somewhat", "not at all", and "I don't know"; "To what extent do you think uvulectomy is harmful or results in a negative effect?" with four choices "very much", "somewhat", "not at all", and "I don't know". The interview also involved some questions on the practice of uvulectomy such as the age of the patients and the amount of money or gifts received from the patients undergoing the uvulectomy procedure.

Analysis

The chi-square test was used to compare categorical variables using the SPSS statistical software package version 10.0 for Windows. The descriptive data and their interpretations were anonymously examined for specific meanings and clustered into meaningful groups. To quantify indications for healing practices and harms of uvulectomy, one common term was labeled in each group. We also used qualitative data by selecting typical expressions in each group to complement or interpret the results of analyses of quantitative data.

RESULTS

Profile of the respondents

Among the 149 traditional healers, 59 (39.6%) were registered healers, 68 (45.6%) were non-registered healers, and 22 (14.8%) were faith healers. As shown in Table 1, there was no significant difference in the age distribution or years of education among the three types of healers. Males were dominant among the registered healers, while more than half of the faith healers were women. The religion of the three groups of healers was significantly different: Muslims made up most of the registered healers, while Christians comprised most of the non-registered and faith healers. Some of the registered and non-registered healers followed other traditional or indigenous religions or beliefs such as Baha'i, Kimbansiste, and Kitawara orthodox religions.

The persons from whom the respondent had learned healing practices also significantly differed among the three groups as follows: about 80% of the registered healers had learned from family members, about 40% of the non-registered from traditional healers, and about 60% of the faith healers from other individuals such as 'inspiration', 'God' and 'clergy persons'.

Traditional healing practices and their indications

As shown in Table 2, all of the registered and almost all of the non-registered healers used herbs for treatment, but only 13.6% of the faith healers did so. Various parts of medicinal plants and trees including fruit peel and sap were used, but the most popular choices were the roots, leaves and flowers of plants and tree bark. The healers mainly prepared concoctions for oral administration from these plants, some of which were used as a laxative to purge the body of 'impure spirits' thought to cause illnesses. Some herbs were ground into paste or powder and then applied to sites affected by fractures, cancer and other illnesses. Concoctions were also used as enemas to cure various illnesses, and paste from herbs was used as a suppository to treat hemorrhoids. Other treatments included steam inhalation of the herbs and application of herbal powder/paste to tattoos or scarified areas. Plant roots combined with tree bark were used for the treatment of post-abortion problems, sometimes by insertion into the vagina.

Most of the healers selected a specific treatment for each illness, sometimes using a combination of treatments and trying an alternative treatment when no results were seen. In addition to herbs, the respondents used bones and other parts of animals, honey, salt, oil and certain types of soil for treatment.

As shown in Table 3, the indications perceived to be treated effectively by traditional healing practices and herbal medicine ranged from acute illnesses such as cel-

	Registered healers		Non-registered healers		Faith healers		*p-value*
	N	%	N	%	N	%	
Total (N=149)	59	100	68	100	22	100	
Age (years)							
20-29	14	23.7	16	23.5	5	22.7	0.459
30-39	16	27.1	26	38.2	9	40.9	
40-49	15	25.5	14	20.6	7	31.8	
50-59	14	23.7	12	17.7	1	4.6	
Sex							
male	43	72.9	40	58.8	9	40.9	0.025
female	16	27.1	28	41.2	13	59.1	
Religion							
Christian	15	25.4	25	36.8	18	81.8	<0.001
Muslim	24	40.7	22	32.3	2	9.1	
others	20	33.9	21	30.9	2	9.1	
Education (years)							
0	10	17	10	14.7	3	13.6	0.6
1-6	18	30.5	26	38.2	11	50	
7-14	31	52.5	32	47.1	8	36.4	
Who taught healing							
family	47	79.7	34	50	4	18.2	<0.001
traditional healer	10	16.9	27	39.7	0	0	
by oneself	2	3.4	3	4.4	5	22.7	
others	0	0	4	5.9	13	59.1	

Table 1. Profile of the three groups of traditional healers

lulites, diarrhea and wounds, to chronic illnesses such as cancer and diabetes. The registered and non-registered healers treated similar indications, but the faith healers also treated other indications related to mental problems and sterility.

The exorcism of evil spirits was practiced by 30.5% of the registered and 25.0% of the non-registered healers, but not by the faith healers. As shown in Table 3, the registered and non-registered healers exorcised evil spirits as a treatment for what they called 'madness' or 'impure spirits', 'invisible' illnesses, sterility, and so forth, in addition to examining such illnesses. The healers who answered 'impure spirits' explained that they caused not only mental but also physical problems.

Prayer was the main treatment procedure, and for some the only treatment procedure, among the faith healers, who explained that their healing prayers were different from the exorcising of evil spirits.

	Registered healers		Nonregistered healers		Faith healers		*p-value*
	N (=59)	%	N (=68)	%	N (=22)	%	
Use herbs							
yes	59	100	67	98.5	3	13.6	<0.001
no	0	0	1	1.5	19	86.4	
Exorcise evil spirits							
yes	18	30.5	17	25	0	0	0.015
no	41	69.5	51	75	22	100	
Ever performed uvulectomy							
yes	1	1.7	6	8.8	0	0	0.088
no	58	98.3	62	91.2	22	100	
Effectiveness of traditional healing compared to modern medicine							
All are more effective	3	5.1	8	11.8	1	4.5	0.028
Some are more effective	47	79.6	41	60.3	15	68.2	
None is more effective	6	10.2	4	5.9	0	0	
Don't know	3	5.1	15	22	6	27.3	
How beneficial is uvulectomy							
Very much	1	1.7	2	3	0	0	0.009
Somewhat	4	6.8	12	17.6	4	18.2	
Not at all	14	23.7	13	19.1	4	18.2	
Don't know	40	67.8	41	60.3	14	63.6	
Do you know the benefits of uvulectomy							
yes	14	23.7	18	26.5	2	9.1	0.235
no	45	76.3	50	73.5	20	90.9	
How harmful is uvulectomy							
Very much	16	27.1	12	17.6	3	13.6	0.013
Somewhat	2	3.4	15	22.1	7	31.8	
Not at all	0	0	2	2.9	0	0	
Don't know	41	69.5	39	57.4	12	54.6	
Do you know the harmful effects of uvulectomy							
yes	13	22	25	36.8	10	45.5	0.074
no	46	78	43	63.2	12	54.5	
Trust modern doctors							
Very much	35	59.3	41	60.3	17	77.3	0.302
Somewhat	24	40.7	23	33.8	5	22.7	
Not at all	0	0	1	1.5	0	0	
Don't know	0	0	3	4.4	0	0	
Cooperate with modern doctors							
Very much	36	61	32	47.1	14	63.6	0.344
Somewhat	21	35.6	27	39.7	5	22.7	
Not at all	1	1.7	5	7.4	2	9.1	
Don't know	1	1.7	4	5.9	1	4.6	

Table 2. Traditional healers' practices and perceptions of healing and uvulectomy and attitudes toward modern doctors

The practice of uvulectomy

Uvulectomy is called 'ELEMI' in Kibembe, the refugees' local language. Only 7 (4.8%) of the healers had ever conducted uvulectomy: 1 (1.7%) of the registered healers, 6 (8.8%) of the non-registered and none of the faith healers; 1 (1.7%) of the 58 Christians, 2 (4.2%) of 48 Muslims and 4 (9.3%) of the 43 other religious beliefs. The above seven healers had performed uvulectomy repeatedly, for a total of 47 cases: one registered healer had treated 2 cases and the six non-registered healers had conducted an average of 7 cases each (range: 2-12cases).

The recipients of uvulectomy were mostly infants and children, starting from a median of 2 months of age (range: 1week-4months) up to a median of 3 years (range: 2-5 years). Five of the healers received 1,375 Tanzanian shillings (TZS) in cash (equivalent to US$1.5 as of May 2001) on average (range: TZS 1,000-2,000; US$1.1-2.2) per uvulectomy, while the other two healers were given a hen (equivalent to TZS 1,400; US$1.6) and a duck (equivalent to TZS 2,000; US$2.2) per uvulectomy.

Perceived effects of traditional healing and attitudes toward modern medicine

Over 80% of the traditional healers believed that 'all or some of the traditional treatments were more effective than modern medicine'. However, 11.8% of non-registered healers believed that 'all the traditional treatments were more effective than modern medicine', while 10.2% of the registered healers believed that 'none of the traditional treatments were more effective than modern medicine'.

Over 98% of all respondents trusted modern doctors 'very much' or 'somewhat'. In practice, 96.6% of the registered healers cooperated with modern doctors either 'very much' or 'somewhat'. However, more than 10% of the non-registered and the faith healers did not cooperate with modern doctors at all or did not know to what extent they cooperated.

Perceived effects of uvulectomy

More than half of the respondents did not know whether uvulectomy was effective or harmful. In all three

	Registered healers	(n)	Non-registered healers	(n)	Faith healers	(n)
Top five indications	fracture	19	fracture	10	madness	7
treated effectively	diabetes	17	diabetes	8	sterility	7
by traditional healing	epilepsy	10	blisters	7	epilepsy	5
	cancer	9	cellulites	6	impure spirits	2
	hemorrhoid	8	diarrhea	6	mental trouble	2
Top five indications						
for herbs	diabetes	22	cellulites	12	sterility	3
	fracture	19	sterility	11	abortion	1
	cancer	16	diarrhea	11	impure spirits	1
	epilepsy	12	fracture	10	mental trouble	1
	cellulites	9	wound	9	paralysis	1
Top five indications						
for exorcising	impure spirits	8	madness	6		
	madness	4	examination	6		
	examination	3	impure spirits	5		
	sterility	2	cellulites	1		
	invisible illness	2	epilepsy	1		
Indications for						
uvulectomy	cough	2	cough	5	cough	3
	vomiting	1	vomiting	4	appetite loss	1
	appetite loss	1	appetite loss	2	throat pain	1
	baby's crying	1	throat pain	2	fever	1
	throat pain	1	baby's crying	1		
	death	1	fever	1		
Harmful effects						
of uvulectomy	death	8	bleeding	15	bleeding	7
	bleeding	7	death	11	death	4
	throat pain/swelling	2	throat pain/swelling	10	throat pain/swelling	3
	nerve injury	1	cough	3	appetite loss	1
			appetite loss	1		

Table 3. Indications for healing practices and the harmful effects of uvulectomy as perceived by traditional healers

groups of healers, those who thought uvulectomy ‘very harmful’ were greater in number than those who thought it ‘very effective’.

Those who considered uvulectomy ‘very effective’ or ‘somewhat effective’ were more likely to be non-registered healers than the others, while those who considered uvulectomy ‘very harmful’ or ‘somewhat harmful’ were more likely to be faith healers.

None of those who considered uvulectomy ‘very effective’ cooperated ‘very much’ with modern doctors, while 25.6% of those who considered uvulectomy ‘very harmful’ cooperated ‘very much’ with modern doctors.

As shown in Table 3, the three groups of healers gave similar answers regarding the indications for uvulectomy, such as cough, appetite loss and sore throat. They described the indications as follows: “Uvulectomy is effective for cough, vomiting, and throat dryness that stops the passage of air or oxygen.” “If uvulectomy is done, patients can then eat food, because a uvula prevents food from passing through throat.” “Uvulectomy can help babies when they have a cough, sore throat or high fever and when they can’t eat.” “If uvulectomy is not done in time, a patient will die because of blocked respiration and swelling of the throat.”

Several responses were observed regarding the harmful effects of uvulectomy among the three groups of healers. The healers pointed out bleeding, death, sore throat, appetite loss, nerve injury, and responded as follows: “I have seen somebody die from cutting the uvula because the patient’s nerve was cut and throat swelling occurred.” “Blood discharge can cause death.” “Throat inflammation and blood discharge can cause death.” “A patient may get thinner, vomit frequently and develop a sore throat.”

Thirteen respondents responded that uvulectomy was harmful only if inappropriately done. “Uvulectomy can be dangerous if the person who does it is a charlatan. The patient may die.” “Uvulectomy is safe if it is done by an experienced nurse in the dispensary.” “When done poorly, the healer may cut the tonsils and the patient may bleed to death.” “When uvulectomy is done poorly, it causes a sore throat and the patient is not able to eat.” “If one doesn’t know how to cut properly, the procedure will hurt the throat and the patient cannot eat.”

Six of the seven healers who had performed uvulectomy in the past responded that the procedure was ‘somewhat effective’ and also ‘somewhat harmful’, and responded as follows: “If one doesn’t know how to cut the uvula, the patient may develop a sore throat. But I have done it in the Congo and had no problems.” “A person may develop a sore throat after uvulectomy. It is dangerous if one doesn’t know how to cut it properly. It is particularly dangerous for babies. I performed uvulectomy in the Congo, but here I do not have the proper instruments.” “If it is done by someone who doesn’t know how to do it, it can cause serious problems.” “A sore throat and bleeding occur after uvulectomy, but there is a type of root which I can use as a medicine to stop such problems.” “One must have sufficient experience but I think there is no problem for adults.” “Uvulectomy affects the throat. If the healer doesn’t know how to cut, it can cause respiratory problems.” The one remaining healer who had performed uvulectomy said, “Uvulectomy is not good at all. Actually it is very harmful. It causes bleeding and death.”

Other surgical practices

Some surgical procedures other than uvulectomy were performed by the healers in the camp, such as male and female circumcision, tattoo, scarification and hemorrhoidectomy.

The female external genitalia, especially the clitoris, and the male hemorrhoid were both called ‘EHANYA’ in the local language. They were described as follows:
“Women with EHAYA cannot get pregnant. So traditional birth attendants cut the EHANYA of women who want to get pregnant.” “EHANYA is cut away with a razor blade or knife. After the operation, traditional medicine is applied to the site.” “It is usually effective, but some people say it is not good because many recipients get sexual diseases.” “About one in 10 women receive this procedure and the results are good. But sometimes it causes severe bleeding and infection from sexual diseases and HIV/AIDS. “

Male circumcision is called “BOTENDE”. “The prepuce of the penis is removed with a machete because in our tribe you are considered to be a child if you still have it.” “Most men receive BOTENDE and it is necessary to win respect as an adult or grown-up.” “It is difficult to do. If you perform it improperly, you will kill many people.”

The herbal application used to treat scarified areas of skin is called “EMBE”. “If someone is suffering from local pain, the skin in the painful region is cut with a razor blade or knife and then the resin of a tree is applied to the bleeding site.” “It cures diseases. But it can also cause excessive bleeding and scaring.” “If one razor blade is used on many patients, it can also cause the transmission of HIV.”

DISCUSSIONS

Uvulectomy was widely performed in African nations such as Tanzania [7-9], Ethiopia [10-12], Sudan [13], Nigeria [14, 15, 16], Morocco [17], Cameroon [18], Niger [19], and in Middle Eastern countries including the South Sinai [20, 21] and Saudi Arabia [22]. Although it was reported as a common practice [5, 12, 23], its prevalence varied among

the tribes, regions and countries [11, 19, 20].

This study demonstrated that various types of traditional healing practices were being conducted for different illnesses in the refugee camp and that most healers were confident with their effectiveness. Among the healers in this camp, however, uvulectomy was not a common practice and only a limited number of registered and non-registered healers had performed it. Uvulectomy was performed on infants and young children by the age of five in this camp, a finding consistent with previous reports [16, 18, 24, 25]. However, in some reports the operation was performed on newborns soon after birth [3, 17].

In our preliminary interview, modern health professionals in the study site suggested that most of the traditional healers believe in the beneficial effects of uvulectomy. However, our results showed that many healers did not know the effect of uvulectomy and that those who thought it harmful outnumbered those who thought it beneficial.

Indications for and adverse effects of uvulectomy raised by the healers in our study were similar to those described in other studies [17, 26, 27, 28]. Moreover, previous studies reported that uvulectomy was performed for its prophylactic and/or curative effect on abdominal pain, insomnia [3] and chronic diarrhea [22]. Other studies reported bronchopneumonia, tetanus, meningitis, sepsis, dehydration, edema of the glottis and cellulites of the neck as complications of uvulectomy diagnosed by modern professionals [3, 10, 13, 29-33].

However, our study indicated that a limited number of healers had repeatedly conducted uvulectomy and that most of them believed it to be effective, although some of them knew its complications. In this camp, special huts were built within modern health compounds for traditional healers to practice their procedures and to collaborate with modern health professionals. However, our preliminary interviews with modern health professionals revealed that not many of the traditional healers had used the huts or engaged in any real collaboration. Our findings showed that the non-registered healers had cooperated less with modern health personnel and performed uvulectomy more frequently than the other healers.

In addition to uvulectomy, healers performed traditional surgical practices such as female genital circumcision and scarification, which had potential complications [34, 35]. However, as was the case in this study site, these risky surgical practices were not regularly surveyed or assessed in refugee camps.

Our study has some limitations. It could not be generalized to other refugee camps because perceptions and practices of traditional healing might differ according to country of origin, ethnicity and culture among the displaced populations. Semi-structured interviews might not be enough to capture all the details of healers' perceptions, beliefs and practices, although this study attempted to gather qualitative information to complement quantitative data.

Access to modern health services has improved, but traditional healing may continue to be a popular or alternative among those who are accustomed to receiving such practices. Especially in emergency or post-emergency situations, modern health professionals might not be well acquainted with the cultures and traditions of refugees or displaced persons who have moved from different places. When severe complications resulting from traditional practices are identified or expected, interventions could include not only directly discouraging such practices but also reducing high-risk procedures, for example by urging people to avoid shared instruments and promoting early contact with or referral to modern health providers.

It might seem easy to just recommend collaboration, but in reality it is difficult to promote real collaboration between modern and traditional health providers. The first step could be precise situation analysis, preferably with the participation of the healers themselves and community people. The second step could be active dialogue and close communication of facts and analysis based on mutual respect.

CONCLUSION

In a Congolese refugee camp in Tanzania, a small number of traditional healers considered uvulectomy to have beneficial effects on cough, vomiting and other conditions, and in practice had performed uvulectomy on infants and young children. However, most of the healers who had conducted uvulectomy were non-registered healers who engaged in relatively little collaboration with modern doctors. An increased child mortality risk due to the adverse effects of uvulectomy was suspected. It is recommended, therefore, that the health authorities of the camp identify the healers who performed this procedure and strive to achieve more strategic communication and collaboration between modern and traditional health practitioners.

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Population Polymorphism of *Trypanosoma cruzi* in Latin America indicated by Proteome analysis and by in vitro amastigote proliferation

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Abstract: Nineteen stocks of *Trypanosoma cruzi* originating from several endemic countries for Chagas' disease in Central and South America were subjected to two-dimensional protein electrophoresis analysis. The presence or absence of a total of 492 polypeptide spots among 19 gel profiles was determined. The stocks were classified into three major distinctive groups derived from (I) Central America and the northern part of South America; (IIa) Central America and the northern part of South America; and (IIb) central and southern parts of South America, which showed perfect concordance with the previously reported classification based on isozyme and DNA sequence analyses. Late log phase of each epimastigote was inoculated to human cell lines WI-38 and Hs 224.T originating from the lung and muscle, respectively, and the number of trypomastigotes released was counted. The number of trypomastigotes from *T. cruzi* in group I released from the two cell lines was significantly higher than that in group III ($p<0.05$). The findings suggested that the phenetic distance appearing within the *T. cruzi* may, to some extent, be associated with the intracellular growth of *T. cruzi*, one of the characteristic features of growth found in the species.

keywords: *Trypanosoma cruzi*; Two-dimensional gel electrophoresis; in vitro amastigote proliferation, genetic polymorphism, Latin America

INTRODUCTION

Chagas' disease, caused by infection of the flagellate protozoan *Trypanosoma cruzi*, is an important health problem in Central and South America, since 16 to 18 million people in the endemic area suffer from this disease and an additional 90 million are exposed to the risk of acquiring the infection [1]. The illness may remain an asymptomatic infection throughout life or develop into overwhelming acute myocarditis in infants or cardiomyopathy in patients in all endemic area and/or digestive forms predominantly in the southern part of South America [2]. Although the factors influencing this variable clinical course have not been elucidated, it has been suggested that the association of clinical symptoms and the severity of the disease in certain geographic regions may be related to genetic factors in the human population and the variability within *T. cruzi* species [2-9].

The extent of genetic variability within the species *T. cruzi* was studied in terms of its isozyme patterns (zymodemes) [2, 4-10], kinetoplast DNA restriction fragments (schizodems) [11], karyotypes [12, 13], and random amplification of polymorphic DNA (RAPD) [8, 14, 15], leading to a conclusion that *T. cruzi* exhibits by nature a high genetic variability. Recently, population-genetic approaches have shown that *T. cruzi* presents a typical clonal population structure and consists of at least two major lineages that are very ancient events [15-17], while analyses of the population of *T. cruzi* isolated from humans and insect vectors in major endemic areas of Central and South America by zymodeme [16, 18, 19] and polymorphism of several genes [20, 21] suggest the existence of three lineages.

In the present study, the proteins extracted from 19 stocks of *T. cruzi* isolated in Guatemala, Ecuador, Peru, Brazil, Paraguay, and Chile were analyzed by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE). The number of shared and unshared polypeptide spots on the profiles among the stocks indicated how many lineages

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Table 1. Origin of the isolates and strains of *Trypanosoma cruzi* used in this study

Name of isolates	Host	Locality	Zymodeme Classification	Reference
172, TM14, TM43, TM47, TM51, TM52,	*T. dimidiata*	Guatemala	I	19, 21
CL strain	*T. infestans*	Brazil	I	21
119	Opossum	Ecuador	I	21
H6, H18	Human	Guatemala	I	19, 21
H1, H20	Human	Guatemala	IIa	19, 21
Peru 1, Peru 2	Human	Peru	IIa	19, 20
Tulahuen	*T. infestans*	Chile	IIb	21
Y strain	Human	Brazil	IIb	21
GS, LO, RF	Human	Paraguay	IIb	18, 19

of *T.cruzi* exist in the endemic areas of Chagas' disease.

MATERIALS AND METHODS

Parasites

Nineteen stocks of *Trypanosoma cruzi* from different ecological and geographic origins were used (Table 1). Epimastigotes of individual *T. cruzi* were maintained at 26℃ in liver infusion tryptose (LIT) medium supplemented with 10% heat-inactivated fetal calf serum (FCS), according to the well-established method [22].

Sample preparation

Epimastigotes were collected by centrifugation (400 x g for 15 min) followed by washing twice with phosphate-buffered saline (PBS). After measurement of the wet weight of the pellet,100 μl of lysis buffer (0.15 M NaCl, 4% Triton X-100, 10 mM Tris-HCl, pH 8.0) containing 2 mM phenylmethylsulfonylfluoride and 20 μg/ml leupeptin was added to 100 mg pellet (approximately 1.5 x 10^9parasites), mixed well by vortex, and kept on ice for 10 min. The mixture was then centrifuged at 8,000 x g for 10 min and the supernatant was collected. An aliquot of the solution was drawn off and was used for the determination of protein concentration using micro BCA protein assay reagent kit (Pierce, Rockford, IL, USA). Finally, 120 mg urea, 10 μl 2-mercaptoethanol, and 22 μl of 40% Ampholine (pH 3.5-10, Pharmacia, Uppsala, Sweden) was added to the supernatant. About 2 mg of protein, which corresponded to 50-80 μl of the final mixture, was used as a sample for 2D-PAGE.

2D-PAGE

2D-PAGE was performed according to the method of O'Farrell [23] with slight modifications. Briefly, the prepared sample was electrofocused in a tube gel containing 9.2 M urea, 2% Triton X-100, and 4% Ampholine (pH3.5-10) for a total of 6875 Vh under the condition that 0.01 M

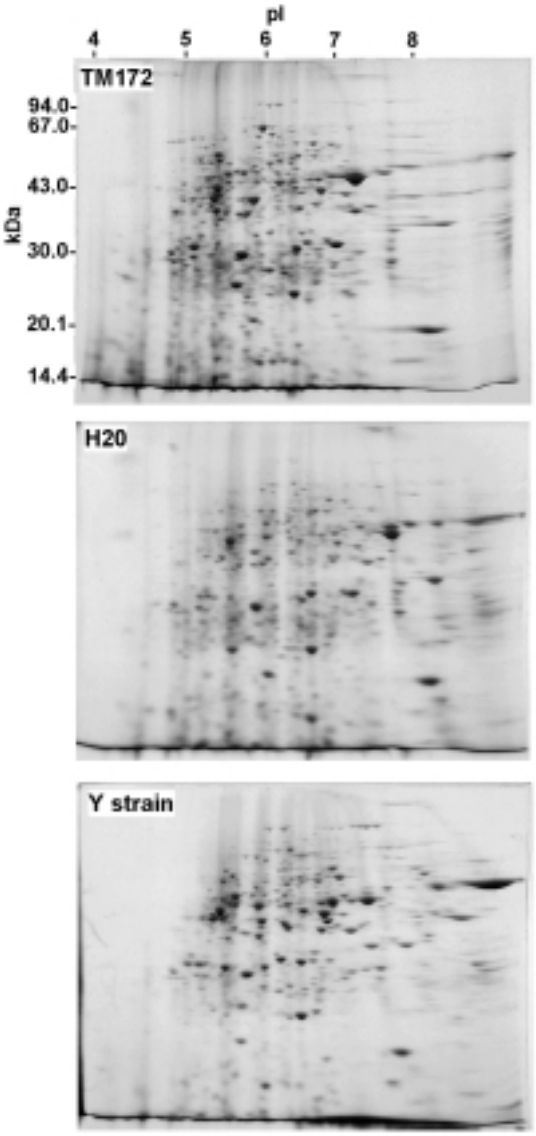

Figure 1. 2D-PAGE profiles of the proteins extracted from 6 isolates of *T. cruzi*. 2D-PAGE was performed as described under Materials and Methods. Nineteen profiles were divided broadly into 3 groups. A representative profile of each group is shown. Group 1; TM172, group IIa; H20, group IIb; Y strain.

H_3PO_4 was used for upper reservoir solution and 0.02 M NaOH for lower reservoir solution. After electrofocusing, gels were removed from each glass tube and were soaked in sample buffer for SDS-PAGE for 1 h. One of the gels was cut into small pieces, soaked in water, and measured pH of the solution. Next, SDS-PAGE containing 11% acrylamide was performed. The gels were soaked in ethanol/acetic acid/water (4:1:5) overnight to remove Ampholine and then was stained with coomassie brilliant blue R-250 for 1 h. After destaining, the gel was dried using Gel Drying film (Promega, Madison, WI, USA). The molecular mass of the

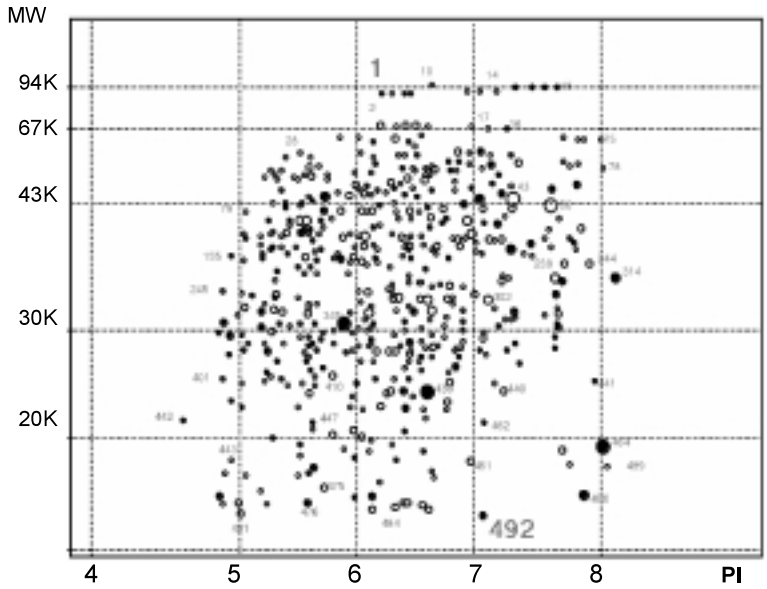

Figure 2. A schematic representation of 492 polypeptide spots. The selected 492 spots were used to judge the presence or absence of the spots among 19 stocks of *T. cruzi*. Part of the spot numbers is shown. Among them, 160 spots (closed circle) were shared among the stocks and 332 spots (open circle) were present in limited stocks.

spots on the gel was estimated using the following standards: phosphorylase b, 94 kDa; bovine serum albumin, 67 kDa; ovalbumin, 43 kDa; carbonic anhydrase, 30 kDa; soybean trypsin inhibitor, 20 kDa; and α-lactalbumin, 14.4 kDa.

Growth of epimatigotes in medium and release of trypomastigotes from human cell lines

Epimastigotes of *T. cruzi* were adjusted to 1 x 10^5/ml in LIT medium containing 10% FCS and cultured in 24-well culture plates (Corning, Corning, NY, USA) for 8 days. The number of epimastigotes in the medium was counted every day at 24 h intervals during culture. The doubling time of each stock was calculated from the number of epimastogotes on day 2-6 showing exponential growth.

Human lung fibroblast-like cell line WI-38 and human muscle rhabdomyocarcinoma cell line Hs 224.T were obtained from American Type Culture Collection (Rockville, MD, USA). The cell lines were maintained in Minimum Essential Medium Eagle (MEME) medium (Sigma Chemical Co., St. Louis, MO, USA) supplemented with 10% FCS (complete medium) at 37℃ in a humidified atmosphere containing 5% CO2.

A suspension of the cells was obtained from the confluent monolayer culture of the cells after treatment of trypsin/EDTA. The cells (1 x 10^5) were cultured with 1 ml complete medium in 24-well culture plate for 24 h. Late log phase of epimastigotes cultured for 8 days was transferred to a 15 ml tube, washed twice with PBS, and finally suspended in MEME medium. One ml of suspension of parasites (1 x 10^6) was inoculated to the monolayer culture of WI-38 or Hs 224.T cells (parasites: cells=10:1) [24]. After 24 h incubation of the co-culture, the *T. cruzi* in the medium and attached to the bottom of the plate were removed completely by several changes of the MEME medium and finally resuspened in 2 ml of MEME medium containing 5% FCS added to each well. The cultures were further continued for 8 days. An aliquot of the medium from the culture was taken off every day at 24 h intervals and the trypomastigotes that appeared in the medium were counted. All cultures were set up in triplicate.

Data analyses

The similarity coefficient (S) defined by Dice [25] was calculated from the number of shared and unshared spots between two stocks among all combinations of *T. cruzi*. The distance (D) was estimated by the following formula [14,26]:

D=1-S=1-2 a/ (2 a+b+c), where

a=the number of spots shared between profile 1 and 2;

b=the number of spots present in profile 1 but absent in 2;

c=the number of spots absent in profile 1 but present in 2.

The unpaired Student's *t*-test was used to determine the significance of differences in the cumulative number of trypomastigotes among the groups. *P* values less than 0.05 were considered statistically significant.

RESULTS

The polypeptide mapping by 2D-PAGE

2D-PAGE analyses of the proteins extracted with 4%

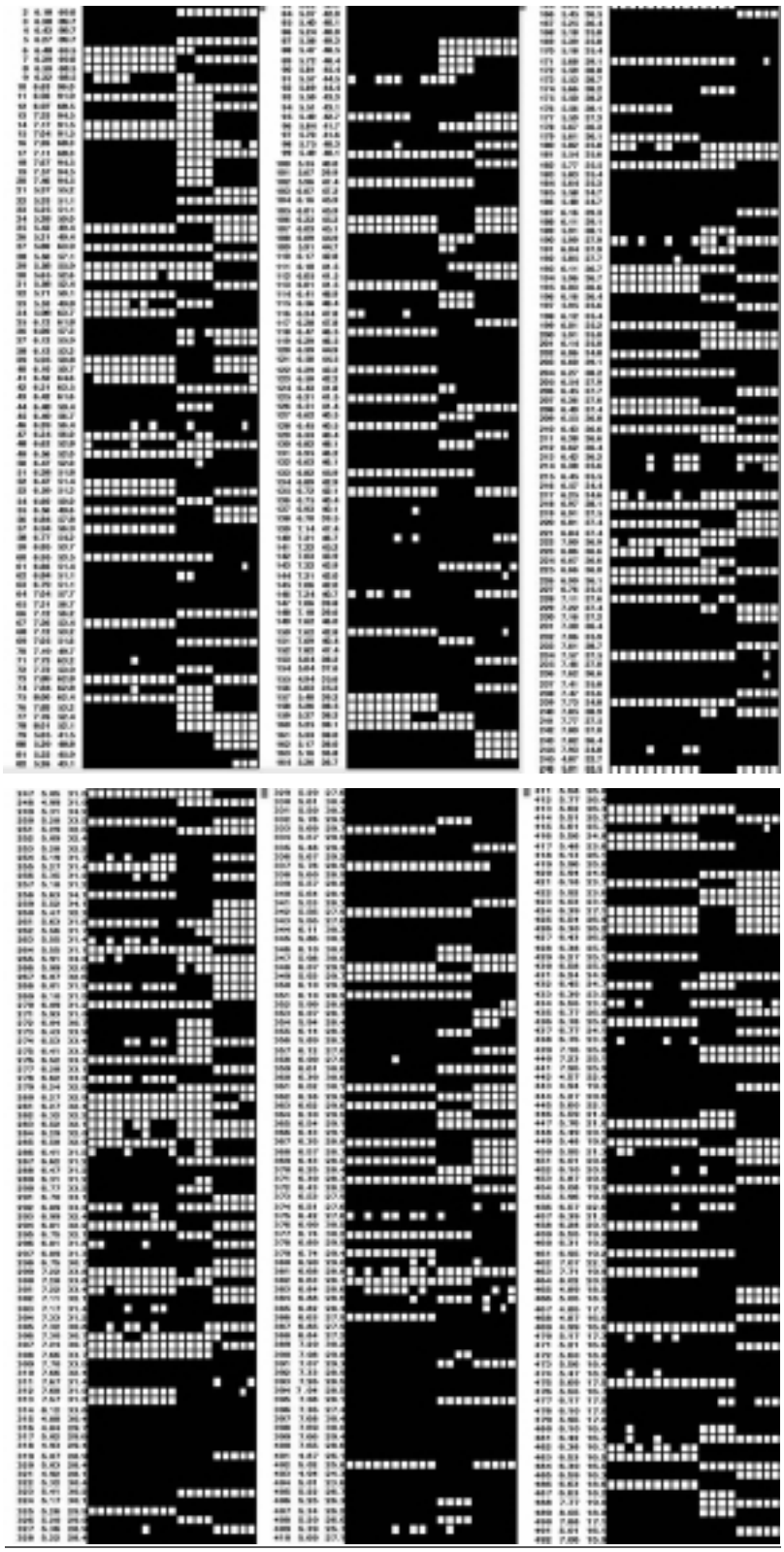

Figure 3. Diagram of the presence (black square) and absence (white square) of 492 spots in individual stocks of *T*. *cruzi*. Polypeptide spots are shown in order of number. Estimated values of p*I* and molecular mass (*M*) are indicated besides each spot number. The isolates are aligned from the left to the right as follows, TM17, TM43, TM47, TM51, TM52, 119, 172, CL strain, H6, H18, H1, H20, Peru 1, Peru 2, Y strain, Tulahuen, GE, RF, LO

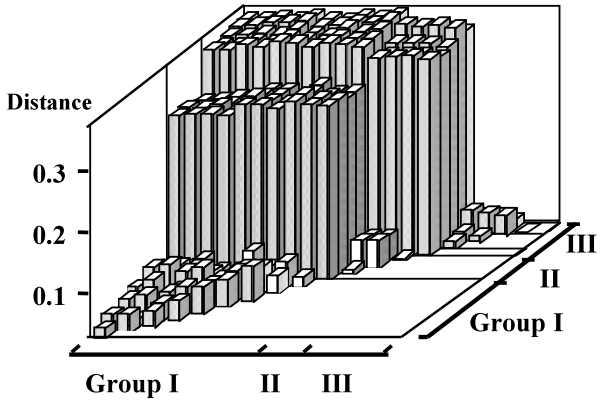

Figure 4. Matrix display of each distance between the two strain combinations from 19 isolates examined.

Triton X-100 from 19 stocks of epimastigotes (Table 1) were examined to evaluate the variability within *T. cruzi* species. Polypeptide spots in the range from 604 to 798 could be detected on individual profiles. Some of the profiles resembled each other, while others were dissimilar. All profiles were then roughly divided into three groups (completely corresponding to I, IIa, and IIb reported by using isozyme profiles [19]) when the gels showing a similar pattern were collected together in one group. Representative profiles of group I (TM172), IIa (H20), and IIb (Y strain) are shown in Figure 1.

Detection of shared and unshared spots of all the stocks

Since it was difficult to determine whether the spots on each profile of two stocks from intergroups were shared or unshared, two of three extracts; TM-43 (group I), H1 (IIa), and Y strain (IIb) or a total of three combinations were mixed and analyzed by 2D-PAGE. By comparison among the three gels, one being a mixture of two stocks and the others were the two individual stocks, it was possible to determine the presence or absence of the spots between stocks (data not shown).

A total of 492 polypeptide spots out of 604 to 798 spots from 19 profiles were selected when the spot could be detected in more than two strains and the presence or absence could be judged among the 19 profiles. A schematic representation of the numbered spots is presented in Figure 2. Among them, 160 spots were shared among the 19 stocks, while 332 were found in limited stocks. The physicochemical properties (relative molecular mass and p*I*) and the presence or absence of individual spots on the 19 profiles are summarized (Figure 3). The sum of the spots detected on the profiles in each stock ranged from 320 (RF) to 358 (H 18).

Based on the presence or absence of the spots, the number of shared and unshared spots between two stocks

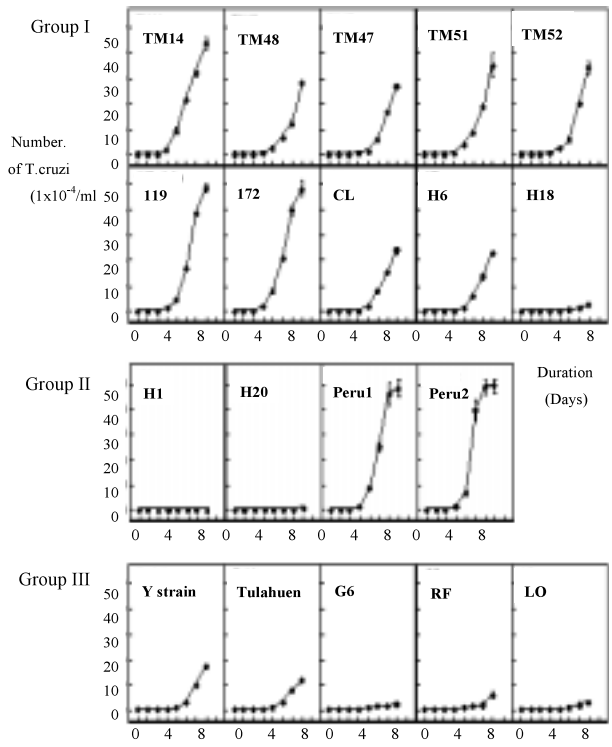

Figure 5. Release of trypomastigotes from human cell line WI-38. Human cell line WI-38 (1 x 10^5/well) cultured in 24-well culture plate was co-cultured with the late log phase of epimastigotes (1 x 10^6) of *T. cruzi* for 24 h. After removal of *T. cruzi* with several changes of culture medium, the cells were cultured for the indicated number of days. The trypomastigotes released from WI-38 cells were counted every day at 24 h intervals.

among all combinations of the 19 stocks was counted. The number of unshared spots was calculated between the stocks and summarized in Figure 4. The results indicate that the 19 stocks of *T. cruzi* can be divided into 3 groups: group I; TM14, TM43, TM47, TM51, TM52, 119, 172, CL strain, H6, and H18: group IIa; H1, H20, Peru 1, and Peru 2: group IIb; Y strain, Tulahuen, GS, RF, and LO. Group I consisted of 10 *T. cruzi*; 8 were isolated in Guatemala, 1 in Brazil and 1 in Ecuador: group IIa 4 *T. cruzi*; 2 in Guatemala and 2 in Peru: group IIb 5 *T. cruzi*; 3 in Paraguay, 1 in Brazil, and 1 in Chile.

Growth of epimatigotes in medium and release of trypomastigotes from human cell lines

The doubling time of epimastigotes of the 10 stocks in group I, 4 in group IIa, and 5 in Group IIb was calculated to be 24.0±0.5 h, 23.7±0.5 h, and 24.0±0.5 h, respectively. The values were not significantly different among the three groups.

The time course of the release of trypomastigotes from WI-38 cells after infection of epimastigotes in the late log

Table 2. Number of released trypomastigotes (day 7) from different cell lines infected by *T. cruzi*

T.cruzi isolates	WI-38 cells (x 10^4/ml)	Hs 224 T cells (x 10^4/ml)
Group I	39.0+23.0*	71.2+24.4*
Group IIa	47.7+47.4	47.2+47.2
Group IIb	9.3+6.9*	26.9+27.0*

*Group I vs IIb $p<0.02$

phase is shown in Figure 5. Trypomastigotes appeared in the medium after four to seven days' culture and thereafter the number increased as a function of the culture day. Similar results were obtained when Hs 224.T cells were used. The number of trypomastigotes released from WI-38 and Hs 224.T cells on day 7 after infection of epimastigotes is shown in Table 2. The release of trypomastigotes in group I was significantly higher than that in group IIb ($p<0.05$). However, within group IIa, two showed a high number of released trypomastigotes while the other two were very low, making it difficult to compare between Group IIa and the other groups.

DISCUSSION

2D-PAGE is widely utilized to detect genetic variations in the population of a species, genus, or family. This method was applied to identify the surface proteins and monitor the change of expression of these surface proteins in the differentiation of epimastigotes to amastigotes and trypomastigotes in *T. cruzi* [27-30]. To our knowledge, this is the first report on an attempt to determine the extent of variabilities of proteins within the *T. cruzi* species.

Among the 492 spots analyzed in this study, 160 spots (32.5%) were shared among the 19 stocks of *T. cruzi* and 332 (67.5%) were present in several limited stocks (Fig.3). The number of shared and unshared spots between two profiles in all combinations of *T. cruzi* was used to characterize the groups (Fig.3). At present, it is difficult to determine whether the extent of variability observed in *T. cruzi* species is high or low, since there have only been reports on the estimation of variabilities by 2D-PAGE in populations in *plasmodium falciparum* (49%, the value being altered to spot unsharing) [31, 32] and *Cryptosporidium pavum* species (3%) [33] in protozoa, *Drosophila* (23%) [34] in insects and the cat family (15%) [35] bear (37%) [36], hominoid primates (40%) [37], and humans (3-6%) [38].

There have been a number of reports concerning the genetic variabilities of *T. cruzi* species analyzed mainly by zymodemes [4-10, 19] and DNA polymorphisms [11-19, 20], suggesting the existence of several lineages of *T. cruzi* within the *T. cruzi* species [reviews: 39-41]. Our observation is essentially consistent with both previous reports and our previous observations [16-21, 41].

As indicated in Figure 3 and 4, there are several identifiable intra-group variations, although the level of the variability is much smaller than that observed between groups. Thus, the prototype of the genome of each group has been well conserved during geographical expansion. It seems there were very few genetic exchange events between groups which might have occurred in the overlapping geographical areas, although the genetic exchange could be detected by the population study [42] as well as in vitro study [43].

The doubling time of epimastigotes of the tested isolates showed no difference, while the number of trypomastigotes released from two human cell lines, WI-38 and Hs 224.T, varied. The number of released trypomastigotes of H 18, H1, H20, GS, LO, and RF was significantly lower than that of the other strains, and the number of trypomastigotes released in group I was significantly higher than that in group IIb ($p<0.05$) (Table 2).

The digestive form of this disease is rare in Central America but frequently observed in South America. It has been suggested that the association of clinical symptoms and severity of the disease with certain geographic regions is related to genetic factors within the species [2-10]. The fact that the isolates of group IIb showed low proliferative activity of amastigotes in vitro might be directly or indirectly related to the pathogenicity of magacolon in South America.

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