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Outbreak Investigation Express

Infectious Disease Outbreaks on a Cruise Ship, April 2011

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Abstract

A cruise ship that docks at Keelung Port regularly began reporting of infectious disease cases in late April 2011. By mid-May, there were a total of 5 varicella, 2 rubella, and 1 mumps cases reported. All ill crew members were recruited from Southeast Asia and joined the cruise during late March to early April. Through treatment, isolation, strengthened disinfection, health monitoring of crew and passengers, and health education, no additional cases were reported by June 17.

During June and August 2010, Hong Kong and Taiwan both reported varicella outbreaks on cruise ships. Because of the current outbreaks of varicella, rubella, and mumps, the cruise company is once again reminded to conduct new crew recruits proper health screening and vaccination, and provide as reference for Health quarantine authorities, to ensure the health of crew and passengers on board.

Key words: cruise ships, infectious disease, cluster, varicella, rubella, mumps

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INSIDE

Original Article

Surveillance, Investigation and Analysis of Human Leptospirosis Infections in Taiwan, 2009-2010

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Abstract

Leptospirosis is a Category IV notifiable or reportable communicable disease in Taiwan. It is a zoonosis and caused by a bacterium commonly referred to as leptospires. The bacterium usually excreted by animal carrier through urination. The bacterium can survive for more than a month in water. The bacterium enters and infects humans when contaminated food or water is ingested or passing through skin wound and mucous membrane when the victim is swimming or playing in contaminated water. In 2009, typhoon Morakot brought a catastrophic flood in areas of southern Taiwan including parts of Kaohsiung County and City as well as Pingtung County and City. Many clustering infections of leptospirosis broke out in flooded areas and had drawn attention of people.

In this study, we analyzed the prevalence of human leptospirosis in Taiwan over the two-year period of 2009 and 2010. We noticed that the leptospirosis infection in Taiwan was significantly higher in males. However, there was no significant difference to the people live in urban or rural area. Also, no seasonal variation was found in the prevalence. Similar to other countries, the epidemiological data suggested flooding problem caused by natural disasters is the primary factor to cause clustering infection of leptospirosis. We noticed that the monthly positive rate revealed by the notification system stayed lower than 10% throughout the period except the three months in 2009 when many clustering cases showed up, with confirmed rates of 18.5~39.7% , after the flooding brought up by typhoon.

Besides continuing leptospirosis surveillance, the health authorities should strengthen the leptospirosis prevention campaign before the typhoon season to alert people and lower the infection risk. Meanwhile, since symptoms of leptospirosis are quite similar to common cold, yet the transmission routes are different, the clinicians should also look into the situation of patient's other family members, the patient's occupation, and residential environment before collecting specimen and reporting the suspect leptospirosis. This shall facilitate sorting the infection source and controlling the endemic, and also avoid wasting of the medical resources.

Keywords: leptospires, positive rate, Taiwan

Introduction

Leptospirosis is a zoonosis that infecting both animals and humans. The pathogen, leptospires, with a length of 6~20μm and extremely active, is a tightly entwined, gram negative bacterium called *Leptospira interrogans*, which belongs to the genus *Leptospira*. So far over 200

pathogenic serovars have been clinically identified and these are further grouped into 25 serogroups due to similarities of serological characteristics [1-2]. Human infection by one serotype of leptospire only generates antibodies for weeks or months. It is generally believed that serovar-specific antibodies are protective and a patient is immune to reinfection with the same serovar. However, antibodies provoked by an infection with a particular serovar do not necessarily protect the person against later infection with different serovars [3]. Human leptospirosis mainly takes place in temperate and tropical climate zones. After infection a patient may display various symptoms such as high fever, headaches, fear of coldness, sore muscles, vomiting, and sometimes jaundice, pinkeye, stomachache, diarrhea, or rash, although some infections may have no symptom at all. When a leptospirosis patient failed to receive treatment, nephritis, encephalitis, liver damages, and dyspnea may occur in severe condition [2].

Leptospira interrogans can infect almost any mammal on earth, particularly murine rodents (including *Rattus norvegicus*, *Microtus arvalis*, *Crocidura russula*, and other murines commonly seen in Taiwan), cattle, pigs, and dogs [3-6]. People at high risk of contracting leptospirosis are simply those who having work or living places exposed to animal hosts. Therefore, high risk groups include farmers, miners, sewer workers, animal husbandry hands or slaughter house workers, veterinarians, and those living in neighborhoods with lesser hygienic standards, typically cohabiting with rodents [7]. *Leptospira interrogans* is mainly excreted by animal carrier through urination or released by decaying tissues of dead animals before it pollutes water sources or soil. In a favorable environment condition of 28~30℃ and pH6.2~8.0, the bacterium can survive in water for more than one month. It can enter human body through skin wounds, or by ingesting contaminated water, or due to swimming or playing in polluted water before contracting infection. Few human to human transmission cases were reported [1]. Because most symptoms displayed by leptospirosis patients are quite similar to diseases caused by other pathogens such as common cold viruses, to ascertain an infection of leptospirosis one has to rely on laboratory testing.

Leptospirosis usually occurs in temperate and tropical climate zones, often seen in places with poor sanitation, or areas struck by natural disasters, flooding in particular [8-10]. Taiwan is located in the subtropical climate belt, sanitation and people's hygienic habits being good, the annual incident of leptospirosis infections used to fall in the two or three-digit range in the past until recently, over 2000 cases were reported a year just in the past few years. To better understand the distribution and prevalence of human leptospirosis in Taiwan during the period of 2009 to 2010, an epidemiological investigation and analysis was conducted. It aims to help the public to prevent the disease, and to provide reference to health institutions for facilitating the making and implementation of disease control policy.

Materials and Methods

The current diagnosis of leptospires in Taiwan is based on the laboratory tests at Research and Diagnostic Center of Taiwan CDC. Two methods, antibody testing and bacterial culture, are performed. Paired sera of acute and convalescent phases are used for antibody testing. A urine or blood specimen is used for culture and identification [9].

Serological Diagnosis

An enzyme-linked immunosorbent assay (ELISA) is used to examine the antibody titers. The acute sample must be taken within 3 days after the onset of the disease symptoms while the second one is collected sometime later than the 14th day after the onset. A suspension of leptospires antigen is added to a patient's serum sample for antigen-antibody reaction. The enzyme-labeled antibody would indirectly transform the reaction process into readable color signals. When the antibody test is positive, a microscopic agglutination test (MAT) will be carried out to confirm the verdict [2, 9]. Since leptospires are not easy to stain, normally a dark field method is used to observe the bacterial particles. Equal volume of live bacterial suspension is used as antigen in MAT, after mixing with serial dilution of patient's serum, allow antigen-antibody reaction to complete, then observe the mixture under a dark field microscope for an agglutination phenomenon to determine the antibody titers in sera samples. A positive case is having a fourfold increase in titer of the convalescent serum. If less than fourfold increase in titer, the patient could be either an acute infection or past infection, the verdict would be inconclusive (or not affirmative).

Cultivation of Leptospires

A blood sample within 10 days after the disease onset or a urine sample taken 7 days post the disease onset from a suspected leptospirosis patient is used for cultivation. Volume 300~500μL of either sample is added into a culture tube with medium. The culture tube is keeping at 28℃ for a period of 12 weeks. One drop of the culture liquid is taken out a week or two, put on a glass slide, and observe under a dark field microscope for leptospires. If no spirochete-like, fast-moving bacteria seen in 12 weeks, the culture result is negative. If fast-moving, fine thread spiral-shaped leptospires are observed, the DNA of the bacteria is extracted and a PCR assay is performed using specific primers. The correct length of PCR amplified segment is then obtained through gel electrophoresis and further verified by sequencing as to ascertain the leptospire culture result.

Results

A statistic analysis was conducted on the data of all reported leptospirosis cases with specimens received at Taiwan CDC laboratory during 2009-2010. Cases for antibody testing were counted on which with the paired serum samples collected, or only one sample was taken due to various reasons including death of the patient. As shown in Figure 1, the total number of suspected leptospirosis cases reported in 2009 was 1,339, the monthly cases in the first quarter (January to March) was 57~80, 72-93 cases in the second quarter (April to June), 108-257 cases in the third quarter (July to September), and 100-160 cases in the fourth quarter (October to December). As a whole, it appears that

more suspected leptospirosis infections were reported in the second half of 2009 than the first half. A closer look reveals that in the second half of 2009 many clustering leptospirosis infections broke out in southern Taiwan, which pushed the reported cases to a new height of 257 in September, more than two times the number of the rest months of the year. As for 2010, the total number of reported cases with specimens received at laboratory was 1,575, and the monthly reported cases in the first quarter were 89~111, 110~125 cases in the second quarter, 157~167 cases in the third quarter, and 134~164 cases in the fourth quarter. It also appears that more suspected leptospirosis infections were reported in the second half of 2010 than the first half, but the difference was not as obvious as in the previous year.

The laboratory test results on suspected leptospirosis cases reported during 2009 and 2010 are summarized as follows: In 2009, there were 204 positive cases among 1,339 cases (positive rate 15.2%), 161 were males and 43 were females, the sex ratio was 4:1. In2010, the positive rate was 4.9% (77/1,575), including 63 males and 14 females, sex ratio was 4:1. Looking into the monthly distribution of positive cases (Figure 2), we noticed that starting from August 2009 many infections broke out in southern Taiwan due to a flood brought about by typhoon Morakot, which raised the monthly positive rate to 18.5%, 39.7%, and 26.3% for August, September, and October, respectively. In comparison, the positive rate stayed within 0%~3.5% from January to March, 3.2%~4.4% from April to June, and was 9.5% and 3.0% for November and December, respectively (Figure 2). As for 2010, the monthly positive rate showed no apparent fluctuations, 2.2%~3.6% from January to March, 0.9%~6.4% from April to June, 3.3%~4.8% from July to September, and 5.5%~9.0% from October to December.

Overall, aside from the clustering infections caused by typhoon, the monthly positive rate was below 10% in other months over the studied period.

Then geographic distribution of the verified leptospirosis cases was presented in Figure 3. The top three counties/cities on the

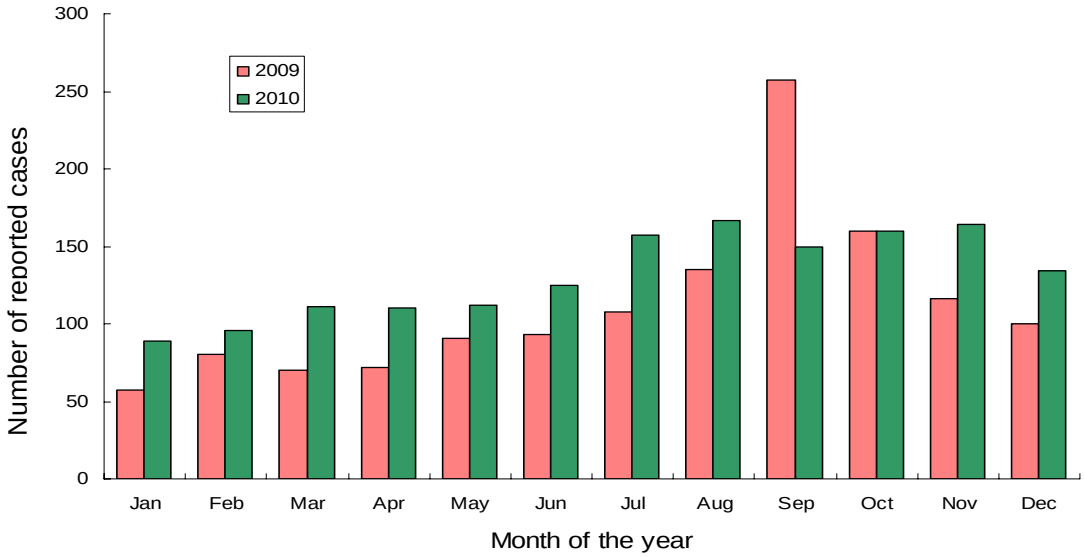

Figure 1. Monthly sums of reported leptospirosis cases in Taiwan during 2009 and 2010 (A case was counted according to the date the specimen was received at laboratory).

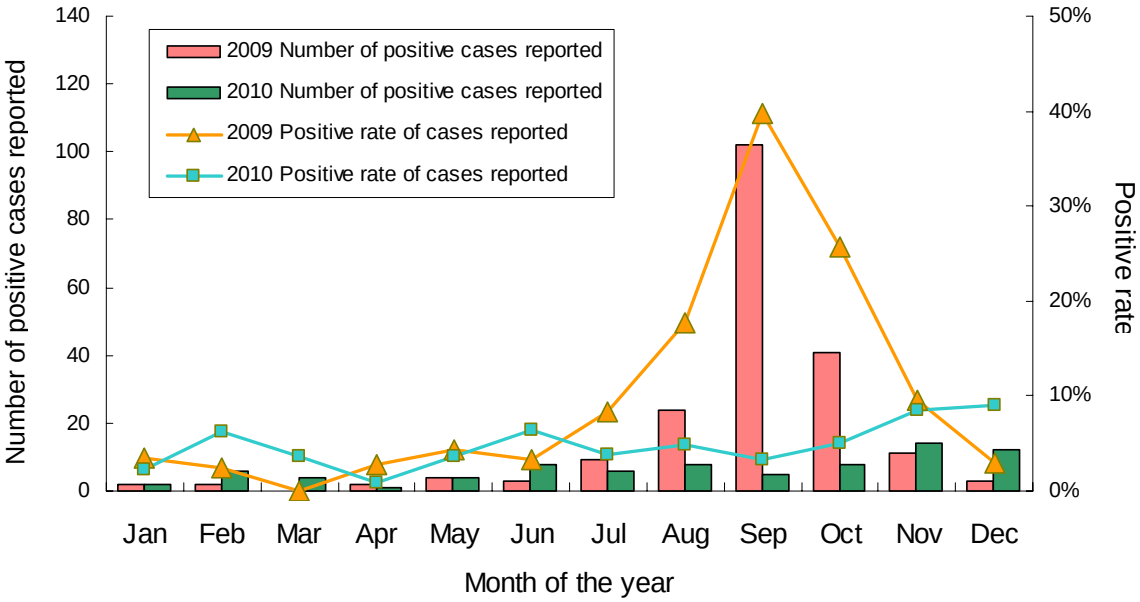

Figure 2. Monthly sums of confirmed leptospirosis cases reported in Taiwan region and their monthly positive rates over 2009 and 2010
(A case was counted according to the date the specimen was received at laboratory).

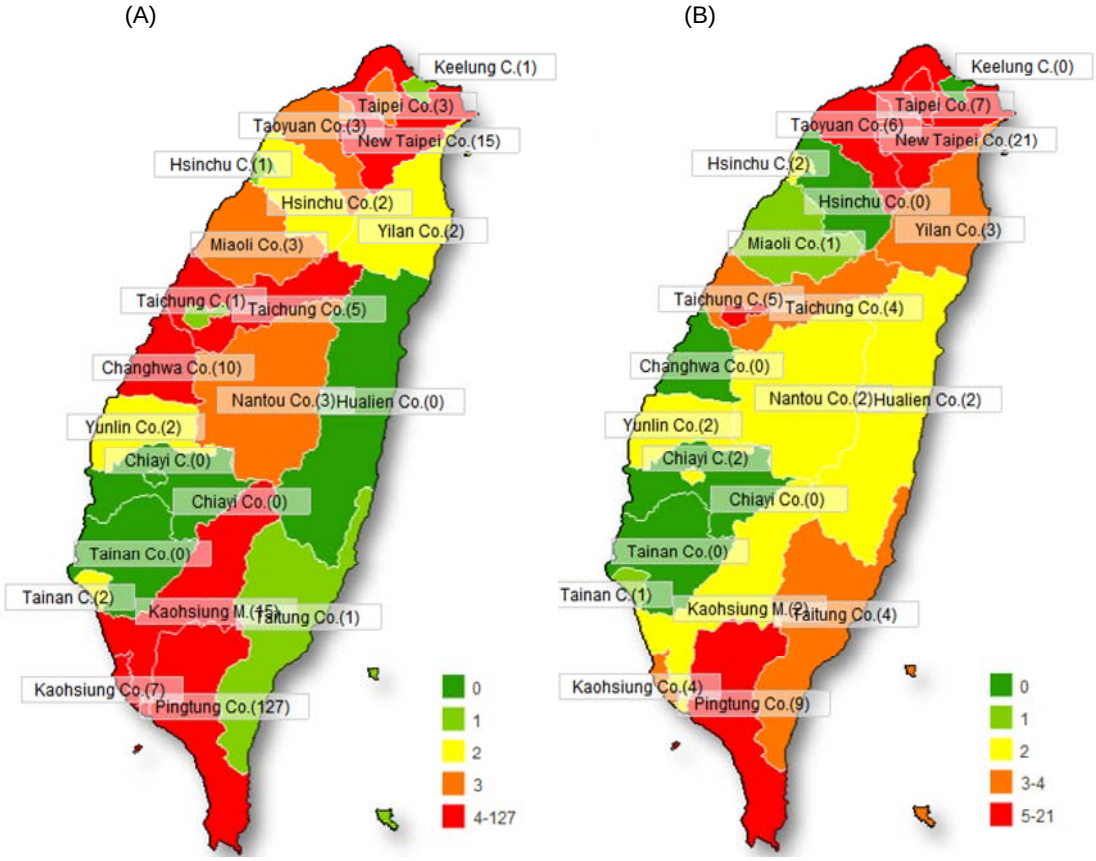

Figure 3. Geographic distribution of confirmed leptospirosis cases reported in Taiwan (A) presents the number of positive cases in each specific locale in 2009, while (B) gives those in 2010
(Data source: Taiwan CDC Databank; Data were based on the onset date, and encompassed the infection sources of the positive cases.)

ranking list having the most leptospirosis cases in 2009 were Pingtung County with 127 cases, Kaohsiung County & City with 22, and Taipei County & City with 18. No case was found in Chiayi County, Chiayi City, Tainan County and Hualien County (Figure 3A). While in 2010 the top three counties/cities having most leptospirosis cases were Taipei County & City with 28 cases, Pingtung County with 10 and Taichung County & City with 8. No case was found in Keelung City, Hsinchu County, Changhua County, Chiayi County and Tainan County. Taipei City & County are metropolitan areas with high population densities, and also ranked in the top three for both years. Kaohsiung County & City, Pingtung County and Changhua County showed the most reduction in case numbers in 2010. On the contrary, the total confirmed leptospirosis cases in Taipei County & City in 2010 was 10 cases more than that of the previous year. Likewise but to a lesser degree, the number of confirmed cases in Hualien County went from 0 to 2 and so did in Chiayi County & City. Taichung County & City stayed at 7 to 8 cases in each year. All off shore islets including Penghu County, Kinmen County and Matsu County had no positive leptospirosis case over the 2009~2010 surveillance period.

Discussion

Comparing the figures of monthly reported and confirmed leptospirosis cases in 2009 and 2010, we noticed number of cases dramatically increased in 2009 between July and September because of flooding in southern Taiwan. Also the number of reported leptospirosis cases seemed to be more in the second half of both years, but there was no significant seasonal difference in terms of positive rate ($p>0.05$). WHO indicated that in countries with high incidence rates of leptospirosis, Indonesia for instance, the prevalent season always overlaps with the local rainy season [10-12]. Although Taiwan has monsoon season in the spring time but it seldom results in severe flooding. Instead, a disastrous flood in Taiwan is usually caused by typhoon. We noticed that in August 2009 typhoon Morakot hit southern Taiwan and brought about a serious flooding in several parts of the Kaohsiung-Pingtung region for more than a week, in turn, which caused the leptospirosis infections inside these areas to a record high of 169 cases from August to October, which accounted for 82.4% of the total cases in a year, and 7.35 times of the confirmed case number in the same period in 2010 (23 cases). This result suggests that the warning from meteorologists on global warming and other climate changes will likely bring about more and more extreme weather conditions and natural disasters should not be overlooked. In other words, Taiwan will face increasing possibility of getting instantaneous heavy rainfalls whenever hit by a typhoon and resulting in severe floods. Therefore, for better effectiveness in disease control, before the arrival of typhoon season each year, it's recommended that the health authorities should, aside from dengue fever prevention

campaign, propaganda emphasizing leptospirosis prevention, also be in order to remind and alert people.

Analysis on the relevance between sex and leptospirosis cases, our data reveals that more males were infected than females in the two consecutive years, with sex ratio of 4:1 ($P<0.005$). This phenomenon is similar to the findings in other southeastern Asian countries, including India (4:1), Thailand (3:1), and Sri Lanka (9:1) [12]. Since leptospires are excreted into the environment by infected animals through urination, people usually get infected after consumption of contaminated water or food, or through the skin wounds or mucus membranes if exposed to polluted water. Work or play in water or soil contaminated with leptospires will become more vulnerable to the infection through contact. Male is at higher risk than female because men tend to work in the field, livestock farm, or a mine where more hard labor is required. Even if women took the jobs, due to much less willingness of being exposed to the sun for example, the female worker is usually willing to wear face mask, gloves, rubber boots for protection, and do a much better and thorough cleaning and preventive job after work. This might be one of the reasons that females generally would not get infection as easy as male workers.

When looked into the geographic distribution of the 2009~2010 positive leptospirosis cases reported in Taiwan (Figure 3), except for the unusual clustering infections in Kaohsiung and Pingtung regions during the 2009 flood incidence brought about by typhoon, the incidence of leptospirosis showed no significant geographic preferences or differences among all counties/cities ($p>0.05$). No difference was found in rural mountainous areas or coastal areas, the agricultural counties/cities also had no apparent more cases than in metropolitan areas. To our surprise, the metropolitan Taipei, which covers Taipei City and Taipei County, reported around 20 confirmed cases per year in both years. This may be related to the habit of civilians in Taipei are more likely to seek for a doctor when not feeling well and the clinicians practicing in Taipei also collect test samples more often than their peers elsewhere. There were 250 more reported cases in 2010 than in 2009, even there was no severe flood situations caused by typhoon. It indicated that physicians are more alert to the leptospirosis infection after the flooding incident. However, even without any severe natural disasters, there were more than 200 cases reported as of the first two months in 2011, only 3 cases (2%) were confirmed after screening by laboratory. Taiwan is not an endemic country of leptospirosis, with several thousand reported cases every year, along with low positive rate, it imperceptibly results in vast waste in medical and manpower resources. To relieve the hardship of this problem and lighten the impact, the medical institutions and health authorities should improve further on heath education and prevention propaganda about leptospirosis, including general public and clinicians as well.

In Taiwan, enterovirus illness is often prevalent in summer, and respiratory viral diseases such as influenza are more common in winter. Many symptoms of leptospirosis, including high fever, headaches, fear of coldness, sore muscles, vomiting, pinkeye, stomachache, and diarrhea are quite similar to those of common cold, so it is quite easy to mix up with the latter. However, the transmission pattern of leptospirosis is distinctly different from that of respiratory diseases. If the clinician has more doubt before collecting the specimen and reporting the case, he can inquire for the information on patient's family members, the nature of the patient's work, and/or the patient's residential environment to better estimate the possibility of a leptospirosis infection. Meanwhile, Taiwan CDC can also provide training to personnel in medical centers, medical societies, and local health authorities, enable them to make the best judgment, so that not only to attain the goal of efficient disease control, and also reduce the waste in medical resources to a minimum.

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Biosafety and Biosecurity

Overview of Infectious Biomaterials

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As biotechnology progressed, varied techniques of molecular biology matured and advanced equipment developed in recent years, resulting in the increase of diverse biomaterials, which are associated with whether in experimental researches, food manufacturing, medical examinations, pharmaceutical development, or industrial products. Biomaterials cover a wide range, including: (1) materials with complete vitality characteristics and are able to replicate directly or indirectly (such as animals, plants, insects, bacteria, fungi, algae, eukaryotic cells, prokaryotic cells, etc.); (2) viruses, organelle, vectors, and so on without complete life patterns; (3) lifeless materials which can be replicated within the hosts and can be isolated (such as blood, cell proteins, antibodies, nucleic acid, etc.).

Some of the biomaterials are hazardous and may lead to human diseases or cause pests and vermination infection on animals and plants, resulting in social and economic losses. In general, biomaterials can be divided into "infectious" and "non-infectious" categories, depending on whether they are infectious to human. According to the definition of Paragraph Four, Article 4 of “Communicable Diseases Control Act” [1], "infectious biomaterials" are pathogens and their infectious derivatives, and are substances that confirmedly contain such pathogens or derivatives. Of which, the "infectious derivatives" signify pathogenic microorganisms even subsequent to artificial hybrid or mutant of genetic material are still contagious, while “substances that confirmedly contain such pathogens or derivatives” refer to specimens from patients with infectious diseases that confirmedly contain some pathogen, or cultured strains of bacteria or viruses identified as some pathogen. Non-infectious biomaterials, such as antibodies, proteins, nucleic acid and so on, are unlikely to cause human diseases. However, some of which like venin, alkaloid, and other biological toxins, may lead to symptoms as inflammation, allergy or toxication due to contacting with human bodies.

As technology advances, the sources and the types of biomaterials have been applied more extensively than before. These biomaterials are contributive to improving human lives with regard to naturally purificated and segregated products, also the cell strains and vectors generated from genetic engineering. However, the utilization of biomaterials also brings us numerous problems. For instance, operating infectious biomaterials may jeopardize the staff’s safety, and misconducting pathogenic biomaterials may cause bioterrorism events; in addition, improper use of recombinant DNA technology could endanger the existence of natural species.

All of the issues shall not be ignored. Hence, application on biomaterials is also regulated by internationally formulating numerous guidelines and regulations [2].

In view of professional inspection and management, the authorities whom are responsible for biomaterials are currently roughly under the command as follows: (1) Council of Agriculture is in charge of whatever that is originated from animals, plants, protected wild animals or related biomaterials; (2) biological agents or biomaterials involving human health are Food and Drug Administration's domain; (3) transplantable human organs and tissues are managed by the Medical Bureau, Department of Health; (4) Centers for Disease Control directs infectious biomaterials that are associated with human communicable diseases, zoonotic microorganisms, and infectious human cell strains. In the case of the genetically modified biomaterials, the National Science Council is presently working on drafting related schemes [3] of management.

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Containment Requirement for Laboratory Biosafety

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The laboratory biosafety levels (BSLs) are designated from BSL-1 to BSL-4 based on the practice code, safety facilities, personal protective equipment, and laboratory equipment. Infectious biological materials shall be handled in the laboratory in compliance with the biosafety level. However, the biosafety level shall be upgraded to the pertinent level, according to the risk factors such as volume, activity, operation, and transmission of the pathogenic microorganisms [1]. The BSL-4 laboratories are strictly limited to highly dangerous pathogens, which lack of effective treatment and preventive measures. Except for the BSL-4 laboratories, the safety requirements for BSL-1 to BSL-3 laboratories are as follows.

BSL-1 laboratories are suitable for handling microorganisms known to be unlikely to cause disease in healthy adults. Examples are *Bacillus subtilis* and *Escherichia coli* K-12. These microorganisms are in the Risk Group 1, or RG1, and have no or low risk to individual and community. BSL-1 laboratories do not need independent spatial structure or special safety requirements, and routine work can be conducted on open bench. Nevertheless, laboratory personnel must equip standard microbiological techniques, conform to the practice regulation, wear personal protective equipment, and wash hands both before and

after the experimental operation [2].

BSL-2 laboratories are suitable for handling microorganisms that can cause disease with moderate risk to healthy adults and low risk to the community. But such Risk Group 2, or RG2, microorganisms can be prevented or treated. Examples are *Salmonella typhi*, Hepatitis B virus, and *Toxoplasma* [3]. In addition to the same requirements of BSL-1 laboratories, BSL-2 laboratories enforce the following requirements: (1) Access to the laboratory must be controlled during the experimental operation. (2) A sign of biohazard symbol must be posted at the entrance of the laboratory. (3) Safety precautions of of needles and other sharps must be made. (4) Disposal regulations must be made to remove and transport the wastes. (5) Biosafety cabinet (BSC) or other protective equipments must be used for preventing from potential infectious aerosols and/or splashes. (6) Autoclaves are available near the laboratory [2].

BSL-3 laboratories are suitable for handling microorganisms that can causes serious or potentially lethal human diseases which can be controlled by preventive or treatment measures. For instance, *Mycobacterium tuberculosis*, *Histoplasma capsulatum*, and HIV belong to Risk Group 3, or RG3. These microorganisms are of high individual risk and with low or moderate community risk. Besides the requirements as same as in BSL-2 laboratories, BSL-3 laboratories require stricter code of practice as follows: (1) Access to the laboratory must be controlled. (2) All wastes must be sterilized and decontaminated before disposal. (3) Laboratory clothing must be decontaminated before sending to the laundry. (4) All manipulation of infectious materials must be conducted within BSCs. (5) Laboratory workers shall wear proper protective clothing, mask, gloves, face shield, goggles, or respiratory protection. (6) Physical laboratory isolation is necessary. (7) Anteroom double doors must equip the function of self-closing and interlocking. (8) Exhaust air is not allowed to be recirculated. (9) Use high efficiency particulate air (HEPA) filters to maintain directional airflow and negative pressure. (10) A hand-washing device should be provided near each exit door [2]. Besides, the current domestic regulations require BSL-3 laboratory to install double-door autoclave for processing all wastes [4].

To do a good job, one must first sharpen one's tools. Laboratory personnel should first assess the hazard of the infectious agents regarding their characteristics and risks, and perform the experiment within the appropriate BSL laboratory. In addition, laboratory personnel should rigorously follow the good microbiological practices in order to prevent accidents caused by human error. In this way, it will be helpful to prevent laboratory infections.

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