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Case report of two imported Brucellosis cases, May 2011

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Abstract

No case infected with the *Brucella melitensis* has been reported in Taiwan over the last thirty years, yet two imported, confirmed brucellosis cases were reported within two consecutive weeks in May 2011. After investigation, it was determined that these two cases had no epidemiologic link. Case 1 was infected when traveling in North Africa, and Case 2 was infected while visiting relatives in Malaysia. The major risk factor for contracting *Brucella melitensis* is ingestion of uncooked beef or mutton, dairy products, or goat milk when traveling in high risk areas of brucellosis. In addition, Case 1 had a history of animal contact in high risk areas of brucellosis. Both cases sought medical care in different hospitals and were reported by physicians. Their specimens were collected and tested by Taiwan Centers for Disease Control and were positive for *Brucella melitensis*.

Key words: Brucellosis, Zoonosis

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Original Article

The overview of development in Smallpox Vaccine

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Abstract

Smallpox is an acute contagious disease caused by variola virus, causing at least two million deaths every year before 1970. After the introduction of smallpox vaccine and the global immunization campaign launched by World Health Organization (WHO), the global eradication of smallpox was achieved in 1980. Smallpox was the first virus to be eradicated from nature by humankind. Nevertheless, the highly contagious and fatal nature of smallpox and the advancement of technology increase the possibility of using smallpox as a bioterrorism agent by terrorist groups. Therefore, many of countries have stockpiles of smallpox vaccine to respond to the threat of biological warfare. In particular, after the 2001 attacks, the United States considered smallpox vaccine as preparedness goods and put significant resources for the advance of production, quality and safety of smallpox vaccine. Although it is not clear whether the actual use of smallpox as a bioweapon would occur or not, we have to be prepared beforehand for risk reduction in bioterrorism threat and damage.

Keyword: Smallpox, smallpox vaccine, second-generation smallpox vaccine, third-generation smallpox vaccine

Introduction

Smallpox used to be one of the most dreadful infectious diseases and the WHO launched a smallpox-eradication campaign in 1967. Under the effort of global immunization against smallpox, the last natural infection of smallpox was occurred in Somalia in 1977 [1-2]. In 1980, the WHO declared the global eradication of smallpox and recommended to stop the smallpox immunization all over the world [2-3]. In Taiwan, the government halted the smallpox immunization since 1979 as a consequence of the remarkable results. Therefore, people born after 1979 are non-immunized group [3].

In 1996, the WHO recommended to destroy existing stocks of smallpox virus before 30th June 1999. Currently, only the Centers for Disease Control and Prevention in Atlanta, USA and the State Research Center of Virology and Biotechnology in Novosibirsk Region, Russia have in possession of smallpox for research purposes [1, 3]. In addition, the last case of smallpox in the world was a laboratory incident involved two medical staffs in Birmingham, UK, 1978 [2], which indicating the concern of quality control in laboratory. Unlike other biological warfare agents, which need equipment for spreading or to be weaponized, the widely dissemination in human and high fatality render smallpox a greatest potential and hazardous biological weapon in the world.

In 2001, a smallpox bioterrorism attack exercise was conducted in the US. It was projected that two months after a smallpox outbreak the country, there might be 3 million infected victims, of which one million will die [4]. Other research shows that after intentionally releasing smallpox, even if it infects only 50-100 persons in the beginning, the infected population will eventually expand by a factor of 10-20 times or more. These estimates are based on a low level of immunity in current population. Smallpox vaccination has been discontinued in the United States since 1972 (except the US military), and nearly forty percent of the US population (approximately one hundred million) have never received smallpox vaccine [5]. In Taiwan, around forty percent of the total population (about 9 million) have never received smallpox vaccine since smallpox vaccination was discontinued. In addition, the immune status of the immunized group is uncertain. An attack by smallpox as a biological weapon or outbreak will certainly pose a great impact on public health and medical system. Consequently, without effective treatment, vaccination plays a leading role on the public health measure and the prevention of disease spreading.

Characteristics of smallpox

Variola virus is a DNA virus which belongs to the genus Orthopoxvirus from the family Poxviridae. Apart from smallpox, there are other orthopoxviruses which cause infection in humans: cowpox, monkeypox and vaccinia [1]. Smallpox is transmitted by airborne droplets and contact with fomites. It is highly contagious, easily cultivated and highly lethal. Survivors usually suffer from long-term complications such as blindness, arthritis, encephalitis, and pockmarks [6].

There are two clinical forms of smallpox. Variola major is the severe form while variola minor is the less severe form. Variola minor is named after mild symptoms and smaller rash, with mortality rates of 1% in non-immunized people. The clinical manifestation of Variola major could be either flat smallpox or hemorrhagic smallpox, both of them are usually fatal and the mortality rates in vaccinated and unvaccinated victims are roughly 3% and 30%, respectively.

Development of smallpox vaccine

Hundred of years ago, healthy persons took pills made from the fleas of cows to prevent smallpox; this is the first recorded example of oral vaccination. Another method to prevent smallpox was to blow powdered scabs of smallpox pustules into the nostrils of healthy persons through a tube. In India, similar measures was practiced by exposing children to material from persons with mild cases of smallpox and inoculating these materials from patients to adult. However, inoculation with live virus is dangerous and ineffective [7]. In 1796, Edward Jenner, a doctor in United Kingdom, discovered that milkmaids did not generally get smallpox after being infected with cowpox. He tested his theory by inoculating healthy individuals with material from a cowpox lesion, which

started a safer and effective practice to prevent smallpox [8]. Around 150 years later (year 1950), the development of freeze-drying technique made the shipping and administration of vaccine more convenient and effective [1].

In the wake of time changing and advancement of technology, smallpox vaccine provides not only eradication of smallpox but also preparedness for accidents or bioterrorism attack. Considering the duration of protection offered by first-generation smallpox and the target groups of vaccination, and addressing the importance of safe, high-quality and efficacious vaccines, second and third-generation smallpox vaccines were developed accordingly.

First-generation smallpox vaccine

The first-generation smallpox vaccine is a freeze-dried live virus vaccine, such as DryVax (Wyeth Laboratories, Inc), the first-generation smallpox vaccine in US. DryVax is a freeze-dried live vaccine and lyophilized prepared from calf-lymph and it does not contain the smallpox virus [9]. The vaccine was first approved in 1931, but Wyeth halted production in 1982. Since the Dryvax license was withdrawn in February 29, 2008, the US started to remove Dryvax from Strategic National Stockpile (SNS) in February 2008 and replace it by the second-generation smallpox vaccine ACAM2000 [10-12].

The seed virus of DryVax vaccine in the US was derived from New York City Board of Health (NYCBH) strain, and other smallpox vaccines were originated from EM-63 strain (USSR), Temple of Heaven strain (China) and Lister or Elestree strain (UK) [8]. Forty countries in the world, including the Netherlands, Denmark, France, Germany, Singapore and United Kingdom have some smallpox vaccine stockpiles, and most of these are first-generation vaccine purchased during the period of the WHO global eradication smallpox campaign [13-14].

Taiwan's stockpiles of smallpox vaccine are from Lister strain freeze-dried vaccine (Figure 1) in UK, which was produced in 1981 or earlier. It is prepared from vaccinia-infected sheep skin. The vaccine is then purified as a yellow or grey-white freeze dried product and later being restored to grey-white suspension with 0.4%(w/v) or lower phenol as a preservative [3]. The vaccine should be inoculated by trained staff with the use of a bifurcated needle (Figure 2). In 2003, research about comparison in immunologic response between different folds of dilution of vaccine was studied in 219 volunteers. This randomized trial was conducted in 97 vaccinia-naïve subjects with vaccine dilution (1:5 or 1:10) and 122 previously vaccinated subjects with vaccine dilution (1:10 or 1:30). The results indicated that all of these groups could induce effective immune response. In Taiwan, we currently have seven hundred thousand doses of smallpox vaccine, and the stockpiles is 28 million doses if calculating by 1:10 dilution with use of a bifurcated needle for 4 persons per dose, which is enough to inoculate the entire population [15].

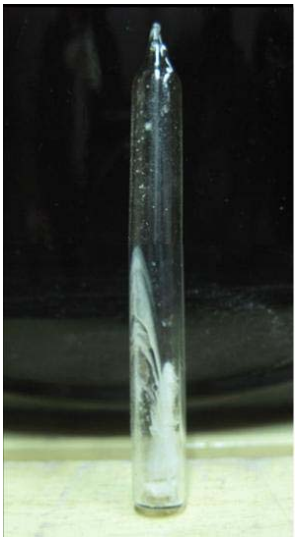

Figure 1. Lister strain freeze-dried vaccine

Figure 2. Bifurcated needle

Second- generation smallpox vaccine

The second generation smallpox vaccine is the freeze-dried vaccine ACAM2000, manufactured by Acambis Inc., a biotechnology company in UK (merged by Sanofi-Aventis, France in 2008). ACAM2000 is derived from a clone of Dryvax (New York City Board of Health strain) and produced using modern cell culture technology, which can produce vaccines rapidly in large quantity and high quality and purity. Both Dryvax vaccine and ACAM2000 are administered with a bifurcated needle and the immune responses they induce are quite similar [16].

Acambis was awarded a contract by the Department of Health and Human Services (HHS) to develop a second generation vaccine since 2000. The vaccines developed from Acambis included ACAM1000 grown in cell cultures of human embryonic lung (MRC-5) cells and ACAM2000 grown in cell cultures of African green monkey kidney (Vero) cells. In February 2003, the US CDC (Centers for Disease Control and Prevention) recommended Acambis to stop the co-development of ACAM1000 and focus its manufacturing and resources exclusively on the production and development program of ACAM2000 based on data in the nonclinical and clinical studies. ACAM2000 was approval by US FDA (Food and Drug Administration) in August 2007. Presently, about 200 million doses of ACAM2000 have been produced in US as the main stockpile of smallpox vaccine [17-18].

Third-generation smallpox vaccine

The third generation smallpox vaccine is IMVAMUNE produced by Bavarian Nordiac, a biotechnology company in Denmark. Imvamune is derived from highly attenuated modified vaccinia Ankara, MVA. The vaccinia virus lost its ability to replicate in human cells and which makes the virus unable to infect others and spread infection. The vaccinia virus was developed from MVA-BN (Modified Vaccinia virus

Ankra-Bavarian Mordiac) strain which had been studied in Germany since 1970 [14, 19]. IMVAMUNE is currently in the phase III clinical trial. It can be administered through intramuscular or subcutaneous injection without using bifurcated needles. This vaccine contains no adjuvant or preservative and the subjects should receive two doses with an interval of 28 days.

The NIAID (National Institute of Allergy and Infectious Diseases) in US began to sponsor the development of the third generation smallpox vaccine in 2003. Imvamune is positioned by Bavarian Nordiac as a vaccine for protection of military and first-line responders individuals contraindicated for conventional smallpox vaccines, such as individuals with HIV (human immunodeficiency virus), people with atopic dermatitis and members of their households. This represents 25% of the general population [20]. The U.S. Department of Health and Human Services (HHS) has awarded a contract to Bavarian Nordic for the delivery of 20 million doses of Imvamune as the stockpile. Imvamune is presently unlicensed by the FDA [18, 21].

Comparison among smallpox vaccines

The research and development of smallpox vaccine in each generation is based on different scientific technology and demand. Although the manufacturing of first and second generation smallpox vaccine differ, both of them present risk of serious adverse events after immunization for individuals with atopic dermatitis, eye diseases treated with topical steroids, immune-deficiency disorders, and pregnancy [22]. Therefore, both the first and second generation smallpox vaccine are considered as conventional smallpox vaccine, while the third generation vaccine, developed for those with high risk of adverse events, is considered as novel smallpox vaccine.

The differences between conventional and novel smallpox vaccine are listed in table 1. The novel smallpox vaccine has advantages of safety and the manner of administration over conventional smallpox vaccine. Apart from the concern of administration in special groups, some studies question the safety of traditional vaccines by reason of serious adverse events such as disability and death after immunization. In 2003, the first generation smallpox vaccine, Dryvax was administered to 37,901 voluntary first-line responders in preparation for potential

Table 1. The comparison between conventional and novel smallpox vaccines

	Conventional vaccine (First and second generation)	Novel vaccine (Third generation)
Name	Dryvax/ADAM2000	IMVAMUNE
Effectiveness	Good	Good
Safety	Not safe in certain groups	Safer based on researches
Administration	More difficult (bifurcated needle)	Easier (subcutaneous/intramuscular)
Dose	One dose	Two doses

bioterrorism events in US. A total of 822 adverse events were reported, and 100 of those were serious adverse events, including 21 cases of myo-/pericarditis and 3 deaths [23].

One study comparing the incidence of myocarditis after immunization showed that subjects injected with Dryvax had a higher incidence of myocarditis than those who received ACAM2000 (10.38 events per thousand compared to 5.73 events per thousand) [16-17, 22]. Another study found that vaccination with the NYCBH strain causes an average of 1.4 deaths per million vaccinations and the vaccination with Lister vaccine causes an average of 8.4 deaths per million vaccinations [24].

For the live attenuated vaccinia virus in the novel smallpox vaccine, the replication cycle in human cells is blocked, which means the virus can neither replicate nor infect. Therefore, the novel smallpox vaccine, which cannot replicate like cowpox virus, does not lead to serious adverse events and secondary and tertiary vaccinia contact transmission [25]. Studies disclose that smallpox vaccination with Imvamune may induce protective immunity with considerate safety, even in subjects for whom conventional smallpox vaccines are contraindicated [14, 19]. The novel smallpox vaccine had been awarded a contract from the US government and it is safer than the conventional vaccine. Nevertheless, the evidence of safety issues of novel smallpox vaccine needs further trials and research since the vaccine has not been approved by the US FDA.

Conclusion

Although smallpox has been eradicated, the real threat of smallpox as a biological weapon warrants due vigilance and preparedness. This threat is recognized worldwide; second and third generation smallpox vaccines have been developed in response. Indeed, the administration of smallpox vaccine remains a crucial countermeasure in the event of a smallpox bioterrorism attack. Taiwan maintains stockpiles of first generation smallpox vaccine (Lister strain), and has sufficient doses to inoculate the entire population. An appropriate public health response to a threat of this magnitude requires a holistic approach with careful planning. Vulnerable populations - including first-line responders, laboratory personnel, and those who are contraindicated to conventional smallpox vaccines - must be accounted for; and, vaccine safety should be closely monitored. Such an optimized vaccine stockpile strategy will protect the public's health, reducing the risk of harm from bioterrorism threats.

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Biosafety and Biosecurity

Management of blind samples in laboratory proficiency testing for infectious diseases

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Most domestic clinical laboratories in medical or health institutions will participate in external proficiency testing annually to ensure the technical capability of laboratory personnel. With regard to laboratory performance for communicable diseases, laboratories take part in proficiency testing activities, either held by domestic organizations, such as the Taiwan Centers for Disease Control or Taiwan Society of Laboratory Medicine, or by foreign organization such as College of American Pathologists (CAP) [1]. Proficiency testing for communicable diseases may incorporate blind samples of infectious or pathogenic samples or strains, raising concerns on safety issues. Regulations regarding their use and transport are discussed.

Title 4, Article IV of *Communicable Disease Control Act* [2] defines “infectious biological materials” as “pathogenic agents of communicable diseases and their infectious derivatives, and substances that have been confirmed to contain such pathogenic agents or derivatives.” In addition, according to *Regulations Governing Management of Infectious Biological Materials and Collection of Specimens from Patients of Communicable Diseases*[2], any changes, including addition, disposal, sharing or storage, of Level 2 and above infectious biological materials shall occur with the consent of the biosafety committee, or the dedicated personnel. As for Level 3 and above infectious biological materials, they shall be reported to the central competent authority for approval in advance. Therefore, people who concern shall differentiate first if the blind samples or strains in the proficiency testing for communicable diseases are “infectious biological materials,” and then make sure if they are subject to the foregoing regulations and to observe them.

Proficiency testing for infectious disease agents includes several common items, such as staining, microscopic examination, culture, identification, susceptibility testing, antibody

testing, and antigen testing [4]. Except for sensitivity testing, samples prepared for other tests may contain non-infectious agents or inactivated agents and may not be defined as “infectious biological materials”. Organizations conducting the proficiency testing are aware of the pathogens in the prepared blind samples. However, if modifications occur, according to the aforementioned regulations, both organizations that conduct or receive performance evaluation, are required to obtain approval from their biosafety committees or the personnel in charge, and thus the “blind” sample system would be meaningless. In addition, although the blind samples for susceptibility test are surely referred to as “infectious biological materials,” it is almost impossible for foreign organizations to follow the regulations mentioned above. Thus, in the event that administrative rules obstruct the operation and improvement of medical laboratories, the use and modification of blind samples and strains in proficiency testing for infectious diseases might be exempted from regulations, while simultaneously following other regulations governing packaging, transportation, and operation.

To guarantee the biosafety of proficiency testing for communicable diseases, domestic institutions conducting the testing shall incorporate in their programs the relevant safety precautions, including the preparation, transportation, and manipulation of blind samples or strains, after granted by their biosafety committees. As for the participating laboratories, in addition to following the safety procedures, all remaining testing samples shall be destroyed after the proficiency testing and be reported to the biosafety committees. Modifications, such as multiplication, sharing, using, research, quality control, and storage of positive samples will be permitted by the biosafety committees ahead of time in accordance with the regulations [2].

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Containment control strategies of laboratory biosafety

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“Laboratory biosafety” refers to preventing the laboratory workers from unintentionally exposure to a harmful environment of pathogenic microorganisms or their derivative, by using the appropriate protection equipments, facility safeguards, along with good microbiological practices. The strategy of laboratory biocontainment mainly includes primary barrier(s) and secondary barrier(s).

The laboratory workers are potentially exposed to the hazardous environments while carrying out the routine laboratory procedure; thus, the relevant biosafety guidelines should be observed to reduce or eliminate the exposure of laboratory workers, other persons, materials, and outside environments from getting infected or contaminated. The primary barrier is applied when work with biomaterials of lower hazardous risk. For example, the laboratory workers should have good microbiological techniques, proper personal protective equipments, and the experiments should be carried out in designated equipment (such as biological safety cabinet, centrifuge safety cups, and sealed rotors). By these methods, workers can avoid infectious aerosols or droplets that can be generated and released. Personal protection equipment includes gloves, protective clothing, masks or facemasks, safety glasses or goggles, and shoe covers. Under special circumstances (e.g. large animal experiments) in which the operation would be unable to proceed in safe and protected facility, then the personal protection equipment will be the last line of defense shielding laboratory workers against pathogenic microorganisms. In some situations, even more protection is needed in the form of N95 masks, overall protection suits, and powered air-purifying particulate respirators (PAPR)

In addition, while the laboratory workers working on the high hazardous risk pathogenic microorganisms, primary barrier alone is not enough for their safety, as the result, there should be a secondary barrier design, which means laboratory safety-based architecture, facility construction, ventilation design, movement direction of personnel and supplies, negative pressure system, air intake and exhaust system, and HEPA filter system, must be carefully considered to ensure the pathogenic microorganisms are all contained in the laboratory rooms, or to reduce the amount of them, prevent the infection spreading to people and the environment inside and outside of the laboratory rooms [1].

The important element of laboratory biosafety is, firstly, to understand the hazardous risk level of the pathogenic microorganisms in working; secondly, use the required barrier(s) and guidelines corresponding to the laboratory biosafety level. In other words, experiments should proceed in laboratory with biosafety level (BSL) that corresponding to the risk group (RG) level of biological materials.

The laboratory biosafety is divided into 4 levels, each level should meet the specific requirements in safety and protection equivalent to the next lower level, plus additional software and hardware in safety and protection designs and standards for laboratory [2]. The director or person in charge of the laboratory should, based on the hazardous risk level and the transmission route of the pathogenic microorganisms, arrange and provide personal protection equipments that suit the safe protection for laboratory workers [3]. Also, the directors or the principal investigators should use risk assessment to determine the appropriate laboratory biosafety level of the pathogenic microorganism, and enhance the necessary laboratory hardware. The criteria for personal protection equipments and hardware of each laboratory level will be described in separate articles.

Overall, an integrated laboratory safety and protection measures, although including the designs of primary barrier(s) and secondary barrier(s), with the concept of “multiple barriers, double backups” is applied, still relies on the good habits and proficiency of laboratory workers eventually. Hence, enhance the knowledge and compliance of personnel by education and training, and an effective auditing and management mechanism are necessary to ensure the safety of personnel and working environments.

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