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Original Article

Retrospection and Prospection for Manufacturing of Snake Antivenins in Taiwan

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Abstract

Taiwan is located in the subtropics, suitable for snake propagation. According to the Department of Health showed, approximately 1,000 people were bitten by snakes each year during 2002 to 2005, mostly by *Trimeresurus stejnegeri*, *Trimersurus mucrosquamatus*, *Naja atra* (common cobra). Injecting the correct antivenin timely is the key treatment to preserve life and can reduce mortality rate to less than 1%. The Centers for Disease Control, Department of Health, Taiwan (Taiwan CDC) have persisted in antivenin manufacturing, research, and development for more than 70 years. Because there are regional differences in snake venin, refining equine plasma from horses immunized by viper venom of native species is needed. Production of antivenin is not easy and it cannot be purchased. Taiwan CDC have entrusted Houli Horse Farm to raise the hyperimmunized horses since 1993, which at one time had about 80 hyperimmunized horses. The horse farm provided good environment for raising healthy hyperimmunized horses and timely production of antivenin in coordination with Taiwan CDC. The horse farm is open to the public for recreation. However, its facility is gradually unable to meet the latest national current good manufacturing practice (cGMP) regulations. To ensure steady supply of domestic antivenin, Taiwan CDC is build a new hyperimmunized horse farm in line with national cGMP requirements during 2009 to 2013, and planned to cooperate with non-governmental organizations or academic institutions to produce antivenin. Through these, Taiwan CDC hopes to enhance domestic horse immunization production technology and the quality of antivenin.

Keywords: antivenin, hyperimmunized horse, Current Good Manufacturing Practices (cGMP)

Foreword

In many tropical and subtropical countries, snakebite is the most neglected public health problem. The World Health Organization (WHO) estimates that about 5 million people are bitten by snakes each year, with 2.5 million by venomous snakes, resulting in at least 100 000 deaths and more than 300,000 people with permanent disabilities [1]. Snakebites occur mostly in Africa, Asia and Latin America, and may cause breathing difficulties, fatal hemorrhagic sepsis, irreversible renal failure, and amputation. Compared with other serious illnesses, appropriate use of antivenins can effectively prevent or reverse the venomous effects of snake bites with high cure rate, avoiding serious consequences such as amputations and deaths. Therefore, antivenins are included in the World Health Organization Model List of Essential Medicines.

Horse-derived antivenom is still the main method of antivenin production because equine blood volume is easy to collect massive plasma and the allergic reaction on human bodies is lowest to horse-derived immunglobulins [2]. Before the 1950s, the Shihlin Branch of the Institute of Tropical Medicine, affiliated to the National Taiwan University, had already engaged in antivenin manufacturing and research. The institute relocated to the present site of Nangang Health Building in 1965, and once renamed “Taiwan Province Institute of Vaccine Research and Manufacturing” and “Institute of Preventive Medicine” historically. In 1991, because the present site was too close to residential housing, horse-rearing were outsourced to avoid affecting the sanitation of surrounding areas.

The commission of hyperimmunized horses livery

Houli Horse Farm is located in Houli District, Taichung City. It was formerly the National Military Combined Logistics Stallion Ranch, used for the breeding horses, improving stallion and training defense cavalry. It has now transformed into a recreation-oriented farm, and is currently the largest and historically most valuable government-operated horse farm. Since 1993, the farm has been commissioned by Taiwan CDC to foster horses through providing two stand-alone, well managed stables. The horse farm provides 39 stalls, each approximately 3.68 m (length) x 3.36m (width) x 3.3 m (height), two special stalls with intensive care facilities, approximately 4.25 m (length) x 4.25 m (width), two open exercise yards, approximately 62 m (length) x 26.8m (width), a class 10,000 cleanroom for plasma separation, a protection facility allowing blood collection from two horses at the same time, two small offices, and two fodder warehouses. There are 45 hyperimmunized horses (Table 1), weighing between 460 to 870 kg in the horse farm, including 39 geldings and six mares. All the horses are from non-epidemic

Table 1. Number and age distribution of hyperimmunized horses

	Age, years (2012)						
	1-5	6-10	11-15	16-20	Total	Currently serving	In recuperation
Number	4	29	11	1	45	27+4*	14

* Four horses are healthy horses for blood collection, providing other experiments which need horse blood, e.g. influenza hemagglutinin inhibition test.

countries imported under “The Horse Importing Quarantine Conditions” specified in the “Animal and Animal Product Quarantine Conditions” announced by the Council of Agriculture (COA), Executive Yuan, and only serve as hyperimmunized horse after having been confirmed as free of disease and parasites. The eight horse handlers hired by the farm are professionals with more than five years of experience in horse-rearing and management. They rotate in three shifts a day year-round to take care of the horses, thereby stabilizing the domestic supply of antivenin products.

As herbivores, horses should eat frequent small meals regularly, and the interval of each meal should not be too long. Therefore, the horses are fed three times daily at 5:00 am, 11:00 am and 5:00 pm. The total consumption of fodder is calculated based on the weight of the horses. The fodder includes alfalfa blocks, corn, cereals, with added Bermuda hay to prevent stomach gas or indigestion which could induce intestinal obstruction in horses. Furthermore, to maintain the health of their bones and hoofs, coarse salt, calcium phosphate powder and vitamin supplement are given to supply the requisite sodium and minerals. The horses are set out to exercise in the yards every morning and after lunch. Staff will take this time to clean the stables, removing manure and repair sharp edges in the facilities to ensure a clean and comfortable living space for the horses. In addition to cleaning up, the staff also need to deworm, clean hoofs, trim hoofs, shave hair, cut tails, and bathe horses to keep the horses clean and avoid the risk of various diseases.

The hyperimmunized horses need to be injected with snake venom regularly to produce antibodies, resulting occasional ulceration, suppuration and scaring on their abdominal skin. , Taiwan CDC highly values horse healthcare based on the respect for life and the protection of animal welfare. We not only commission veterinarians in animal hospitals of national universities to give horses annual health examinations, but also provide monthly health check, strengthen skin care. The horses are weighed every six months, have enhanced observation of their nutritional status, timely supplementation of hay and nutritional supplements such as vitamins and minerals, as well as testing their blood biochemistry before experiments. If horses become sick or emaciated, veterinarians would examine and provide treatment as soon as possible, to understand the causes and to improve the horses’ condition. Moreover, COA or Taiwan CDC’s Committee of Experimental Animal Care and Use will send staff to inspect the management of horses annually for continued promotion of humane animal management and improving the facilities. Because the experimental hyperimmunized horses are not suitable for public visits, Taiwan CDC initiated the mural project (Figure 1), to separate the two stables from tourist routes, thus reducing disturbance to the hyperimmunized horses by tourists.

Antivenin production

Antivenin products in Taiwan were produced during the Japanese Showa era. The antivenin manufacturing technology used Tanaka’s water-based immune technology.

Figure 1. Houli Horse Farm built a mural

Untreated minute to high doses of snake venom were injected into horses daily. The immunization period was up to 140 to 180 days, but was prone to failure, especially for the production of *Naja atra* and *Bungarus multicinctus* antivenin. Because of the venom's strong neurotoxicity, without precise scales to measure small amounts, horses would sometimes be poisoned and die. Some horses suffered from intestinal peristalsis nerve damage and had slowed or no peristalsis following injection of untreated neurological venom, leading to the food stagnation and chronic abdominal dilatation, even acute intestinal occlusion, resulting in death. Then, high potency serum could not be obtained. At that time, the staff in charge of the production phlebotomized horses to obtain horse-derived antivenom sera. Four to five assistants held sterilized volumetric cylinders to fill with horse blood. After letting the cylinders standstill for three to four hours, the iron ball clipped into the volumetric cylinders and squeezed the coagulated blood out to separate from serum. The antivenin was then packaged and put on the market without having purified or sterilized the antivenin. In 1966, the process was amended, adding anticoagulant, sodium citrate to blood, and then obtain the serum after sedimentation. The leftover blood cells, were mixed with normal saline and nutriment, and transfused back to the horses. This prevented the horses from over phlebotomy and early languishment, and extended the horses' service time. In the 1970s, with more patients bitten by snakes, the antivenin supply was often inadequate. To resolve the antivenin shortage, the Institute of Preventive Medicine was established in 1975, followed by the establishment of Horse-Derived Antivenin Research Team established in 1978. Through the efforts of Director Chao-hsiung Yang of the Institute of Preventive Medicine and members of the Horse-Derived Antivenin Research Team, after hundreds of experiments to make improvements, the current protocol used glutardialdehyde to pretreat snake venom, and produced snake venom toxoid [3]. Adjuvant was and fully mixed with equal parts of venom to hyperimmunize horses. The titer of horse antiserum in vivo increased from 60 units to 120 units [4]. Serum refining method was

modified using pepsin digestion, and extracting only the immunoglobulin from sera greatly increased the titer six to eight times. Serum products were preserved as lyophilized crystals instead of in liquid form, prolonging the storage period from two years to five years, effectively solving the long-term problem of antisera shortage. The antivenin now can be adequately supplied to various medical facilities countrywide. According to literature review, before the antivenin products were widely available in Taiwan, mortality rate of snakebites was 6.27%; it has now reduced to less than 1% [5].

Four antivenins produced by Taiwan CDC include: "Antivenin of *Deinagkistrodon acutus*", "Antivenin of *Bungarus multicinctus* and *Naja naja atra*", "Antivenin of *Trimeresurus mucrosqamatus* and *Trimeresurus gramineus*", and "Antivenin of *Daboia russellii*", covering Taiwan's six major vipers. Each year, approximately 3000 doses of antivenin are used, saving approximately 1000 patients from snakebites (Table 2). Currently, only 22 pharmaceutical companies in 17 countries, including the United States, France, Germany, and Japan, are capable of producing antivenins. Companies capable of producing snake antivenin are internationally renowned pharmaceutical companies and the Vaccine Center of Taiwan CDC. The Vaccine Center has worked with experts of the World Health organization and agencies in Japan and the Netherlands regarding production technology, and has continued to improve quality and developing new products. Among these, "Antivenin of *Daboia russellii*" won the Service Award of the 3rd "Rare Diseases Drug Supply, Manufacturing, Research and Development Incentive Program" in 2004, and obtained drug license from the Bureau of Pharmaceutical Affairs, Department of Health in 2008. In addition, since 2003, Taiwan CDC has successively received more than 30 orders from zoos, fire stations and wildlife protection associations in the United States, United Kingdom, the Netherlands, Germany, Malaysia, Hong Kong and Israel (Table 3). The amount of sales exceeded NT$ 4 million.

Table 2. Annual sales of domestic antivenin products (Units: Dose)

	Year								
Product	2003	2004	2005	2006	2007	2008	2009	2010	2011
Lyophilized Antivenin of *D. acutus*	270	351	363	605	142	583	341	188	146
Lyophilized Antivenin of *Tr. Mucrosquamatus* and *Tr. gramineus*	2,984	3,248	2,901	1,991	2,676	3,023	3,066	3,971	3,037
Lyophilized Antivenin of *B. multicintus* and *N. atra*	986	1,518	1,541	1,510	1,060	1,591	1,344	1,337	1,176
Antivenin of *D. russellii*	-	-	-	-	-	-	75	6	11

Note: Antivenin of *D. russellii* became available on market on May 1, 2009.

Table 3. Annual overseas purchases of antivenin products (Units: Dose)

	Year								
Product	2003	2004	2005	2006	2007	2008	2009	2010	2011
Lyophilized Antivenin of *D. acutus*	55	15	15	40	0	35	10	5	25
Lyophilized Antivenin of *Tr. Mucrosquamatus* and *Tr. gramineus*	35	6	26	0	35	67	0	16	30
Lyophilized Antivenin of *B. multicintus* and *N. atra*	40	22	20	50	0	22	10	1	10

New Pingtung Hyperimmunized Horse Farm

Because the facilities Taiwan CDC rented from Houli Horse Farm do not meet cGMP requirements, Taiwan CDC planned to collaborate with schools or organizations with breeding and managing hyperimmunized horse know-how (organizations with at least livestock farms, animal hospitals, animal husbandry, veterinary, and biotechnology departments) or to commission out the operation of improving horse hyperimmunization technology , horse care and management, breeding high antibody titer horses, and establish the operational mechanisms to effectively enhance domestic technology of horse hyperimmunization and quality of antivenin production. Therefore, under the order ratified by the Executive Yuan, Taiwan CDC transacted the “plan of establishing new hyperimmunized horse farm” on August 14, 2009, and chose to build the first hyperimmunized horse farm in line with cGMP regulations in National Pingtung University of Science and Technology (Figure 2). The construction area of this plan was about 9,934 m^2, comprising of six stables, two forced exercise yards, three outdoor athletic fields, rooms for trimming hoofs, and a clean room for plasma separation that complied with cGMP regulations (Table 4). The new hyperimmunized horse farm was planned based on five basic international principles of horse farm settings to ensure animals are free from hunger, thirst, discomfort, pain, and fear, and display normal behaviors. The new facility will be able to house up to 50 hyperimmunized horses and 25 breeding horses, providing horse plasma for annual production of 5000 refined antivenins, steadying domestic antivenin supply. At the same time, antivenin production technology transfer to private enterprises will be conducive to training personnel, supporting domestic industries, and producing antivenins for other countries to expand multinational friendship.

Figure 2. The first hyperimmunized horse farm in line with cGMP regulations in Taiwan

Table 4. Comparison between Pingtung hyperimmunized Horse Farm and Houli Horse Farm

	Facility	
Setting	Pingtung Hyperimmunized Horse Farm	Houli Horse Farm
Plasma separation room	Class 100 – 10,000 clean room (In accordance with international PIC/S GMP criteria)	Class 10,000 clean room (Unable to comply with cGMP requirements)
Stalls	One big horse per stall	Two big horses per stall (would be difficult to determine food intake by each horse)
Stable flooring	International class materials to reduce the incidence of laminitis	General stalls: cement floorIntensive care stall: rubber mats
Air conditioning in stables	Wet pad cooling and ventilation system	Natural ventilation
Forced exercise yard	Two (approximately 20 m × 60 m) To enhance horse immunity by exercising	Not available
Outdoor athletic field	Three (approximately 60 m × 60 m)	Two (approximately 62 m × 26.8 m)

Conclusion

Taiwan CDC is currently the only producer of antivenin products in the country. Since the Japanese colonial period, we have made antivenins for more than 70 years with annual output of about 5000 doses. The product quality has been affirmed. In past five years, only three persons died of snakebites because of delays in seeking medical treatment. Because viper venoms differ around the world, therapeutic antivenins have to be derived from native vipers. However, lots of countries do not have the technology of antivenin production. Each year, approximately 125,000 persons die of snakebites because of the lack of effective treatment, highlighting the importance of establishing local production technology. Taiwan CDC will continue to supply high quality biological products to protect the public's health, and will transfer the technology to private enterprises with willingness and hope to export our products to countries in need and save more lives.

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Outbreak Investigation Express

Indigenous Dengue Fever Outbreak Caused by Importation at Luchu Township, Taoyuan County

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Abstract

A medical center in northern Taiwan notified a suspect case of dengue fever of a 33 years old man on September 19, 2012. The virus is different from the virus strains in southern Taiwan, but similar to a Cambodian strain in 2011, though the case had history of travelling to southern Taiwan. Epidemiological investigation revealed that the case had stayed at Taoyuan County during incubation and viremic period. Furthermore, two family members were found to have suspected symptoms during middle and late August, and were then confirmed as dengue fever. Further investigation found the relatives at neighbor had been touring to Cambodia in late July. Within three days after returning, one of the children had fever and diarrhea, and was confirmed by laboratory as the first case of imported dengue fever and thus caused the outbreak. A total of nine cases were confirmed as indigenous dengue fever infection. The outbreak was attributed to many factors including delayed diagnosis, delayed reporting, living in remote area, vacant lots, vegetable garden, and abandoned pig house with piled junks around the residence, and with level 9 container index, meaning high density of dengue vector. Since receiving the notification, the public health authorities immediately mobilized the manpower and other resources for disease control, and have taken integrated strategies including surveillance of mosquito breeding sites, environment sanitation, vector source reduction and chemical control for mosquitoes. Within a week, the authorities removed large quantity of positive vector containers and potential ones, rapidly reduced the vector density, and no further infection was noted.

Keywords: dengue fever, outbreak, imported, indigenous

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