防疫第一線的基因科技

全球第一支次單位藍耳病疫苗體現創新抗病毒策略

Gene Technology at the Frontiers of Disease Prevention

The World's First Subunit Vaccine Against Porcine Blue Ears Disease

圖片來源：基因線上提供

全球動物疫苗市場估計在2015年已突破100億美元，到2025年更預計會倍增至200億美元左右，以牛、豬、和雞疫苗居大宗。2016年全球豬隻疫苗約有17.84億美元的產值，對於防範豬隻疫病和降低畜牧損失實在功不可沒。不過病毒、細菌、寄生蟲等防疫對象也不會善罷甘休，尤其像豬隻最常見的疾病如口蹄疫 (foot-and-mouth disease, FMD)、俗稱「藍耳病」之豬生殖與呼吸道綜合症 (porcine reproductive and respiratory syndrome, PRRS)、病毒引起之豬流行性下痢 (porcine epidemic diarrhea, PED)、經典豬瘟 (classical swine fever, CSF)、和豬流感 (swine flu) 等，均屬於重組和突變性極高的RNA病毒，因此疫苗也須隨病毒的演化持續改良方能維持效力。傳統疫苗製法是採用致病病毒，將其去活性成為不活化疫苗(inactivated vaccine)，或是降低其毒性製成活病毒減毒疫苗 (live attenuated vaccine)。但在面對RNA病毒時，流行病毒株可能與不活化疫苗中的病毒差異甚大，疫苗也就無法發揮保護效力；而活病毒減毒疫苗則有可能在施打後發生與野外毒株或流行毒株重組的風險，讓防疫工作適得其反。所幸基因科技近年的興起也帶給疫苗研發許多新的思維和工具，對產業發展具有重大意義。

The global animal vaccine market is estimated to have surpassed 10 billion US dollars in 2015, and is looking up to break 20 billion US dollars by 2025. Vaccines for cattle, swine and chicken make up most of the pie. By 2016, swine vaccines have produced a market value of 1.78 billion US dollars. This indicates countless herd loss and economical damage by infectious diseases are prevented. But these nasty pathogens aren't so easily dealt with, viruses of common swine diseases like foot-and-mouth disease (FMD), porcine reproductive and respiratory syndrome (PRRS, also known as the "blue ears" disease), porcine epidemic diarrhea (PED), classical swine fever (CSF), and swine flu are all evolving at a rapid rate, constantly reforming their RNA composition. Vaccines will also require continuous improvement to match the ever-evolving threat. Traditional vaccines are made through "deactivating" the pathogen to create "inactivated vaccines", or simply "tuning down" the toxicity of the pathogen to create "live attenuated vaccines". But these methods are less effective when dealing with RNA viruses, where the prevalent strain may have major differences with that of the inactivated vaccine, or perform recombination with the live attenuated vaccine strains, enhancing evolution rather than preventing the disease. Thanks to the rise of technology in molecular biology, new strategies for vaccine development are sprouting every day, revolutionizing the industry.

藍耳病的防疫難題

約莫20年前，口蹄疫是最令豬農聞之色變的疫病，迄今在世界各地已合計造成數千億美元的損失和上千萬豬隻被撲殺的衝擊。不過從90年代中期開始，俗稱「藍耳病」之豬生殖與呼吸道綜合症(PRRS)悄悄成為豬農最關注的疫病，目前也是美國和歐洲經濟衝擊最大的豬隻病症。研究模型預測嚴重的PRRS感染可對單一大型豬場(1000頭母豬的規模) 造成高達65萬歐元的損失，相當驚人。儘管如此，PRRS的防治在過去一直仰賴具有與野外毒株重組風險之活病毒減毒疫苗，因為不活化疫苗保護效力有限，即使對於和疫苗成分一模一樣的病毒也無法完全保護。面對傳統疫苗的限制，各界轉而投入以基因科技為基礎的重組疫苗(recombinant vaccine)，包含採用病毒特有蛋白質和其他免疫促進蛋白質的次單位疫苗(subunit vaccine)、可表現病毒抗原的DNA疫苗、或利用載體加強免疫反應之載體疫苗等。近來陸續有研究嘗試使用DNA疫苗、轉殖植物產生之病毒蛋白疫苗、以及無法複製之活病毒疫苗，但其疫苗成本太高及在豬隻的保護效力均不佳。

The Challenges of Blue Ears Disease

About 20 years ago, the foot-and-mouth disease was the most feared disease among swine farmers, causing hundreds of billion US dollars worth economic loss and the elimination of tens of million pigs. But starting from the mid 90's, PRRS, commonly known as "blue ears", made its mark among swine farmers. It is also currently the most damaging porcine disease in European and North American regions. Research simulations estimate that a serious infection in an industrialized farm (scale of >1,000 sows) can generate a devastating loss up to 650,000 Euros. Even in the constant threat of PRRS, prevention methods against the disease are still limited to inactivated & live attenuated vaccines, despite the lack of potency and the risks of creating a recombinant strain. Even vaccines created with wild strains can't provide ideal protection. Faced with such restraints, the industry turned to develop recombinant vaccines that heavily rely on gene technology. Subunit vaccines that carry virus-specific proteins or other immune-stimulating proteins, DNA vaccines that present the antigen of the virus, or Carrier vaccines that enhance the host's immune response. Yet in recent years, many projects revolving around DNA vaccines, botanical recombinant vaccines or irreproducible live virus had been proven to be either too expensive or simply lack potency.

圖片來源：基因線上提供

來源：瑞寶基因提供

嶄新的抗病毒策略在首支核准上市的藍耳病次單位疫苗得以實現

不過在2017年，由韓國首爾國立大學(Seoul National_University)獸醫病理學系之團隊針對一項新型PRRS次單位疫苗所進行的研究顯示，在21-42日齡之豬隻施予該疫苗後，再於63日齡投予PRRS第1型(PRRSV-1，北美株)或第2型(PRRSV-2，歐洲株)病毒，則有施打疫苗之豬隻的體溫、肺臟、病毒量、和免疫等表現均優於未施打疫苗之豬隻。2018年在韓國進行的田間試驗顯示，受孕母豬分別在懷孕期間第58天和第79天施予該疫苗後，流產率相較於未接受疫苗之母豬下降64%，每窩平均離乳仔豬數則提升37%。有趣的是，韓國這兩項研究所探討的疫苗，正是由台灣廠商瑞寶基因研發之 PRRSFREE® 次單位疫苗，2012年於台灣取得藥證上市成為全球首支 PRRS 次單位疫苗。PRRSFREE® 疫苗的特色是活化豬隻本身的免疫反應以有效打擊_PRRS病毒，原理是將去毒性之綠膿桿菌(*Pseudomonas*)外毒素載體與PRRSV-1和PRRSV-2之開放閱讀框 (open reading frame, ORF) 產物ORF1_ORF5_(PRRSV膜蛋白)、ORF6_(PRRSV膜蛋白)、和ORF7(病毒殼蛋白)結合，利用大腸桿菌表現並純化重組蛋白，讓這些次單位蛋白抗原得直接進入攸關免疫反應強弱之樹突細胞，進而強化免疫反應並產生免疫記憶。

在台灣的田間試驗結果顯示在分娩前8及5週使用疫苗後，順利分娩的比例提高25%，每窩平均仔豬數提升1.5頭，成效良好。目前瑞寶基因正致力擴增 PRRSFREE® 疫苗成為雙價或多價疫苗，同時也將此次單位疫苗技術運用在豬環狀病毒2_(porcine circovirus-2, PCV2)、CSF等疫苗的開發，未來更準備研發PRRS+PCV2+SEP三合一次單位疫苗。

A Novel Strategy to Fight Viruses was born with The Success of PRRS Subunit Vaccine

In 2017, a veterinary pathology team from Seoul National University conducted a field test on a novel PRRS subunit vaccine. Results show piglets inoculated at 21-42 days age with the subunit vaccine, and boosted again with the PRRSV-1 (North American strain) or PRRSV-2 (European strain) vaccine at 63 days have better condition in body temperature, lungs, total virus count and immune responses than unvaccinated piglets. In 2018, a Korean field test indicated abortion rates decreased by 64% in pregnant sows that received this vaccine on the 58th & 79th day of pregnancy to those that did not, and the total amount of survived weaning piglets in each litter increased by 37%. To be noted, the vaccine discussed in these two Korean studies is the PRRSFREE subunit vaccine developed by the Taiwan biotech company, Reber Genetics. It became the globe's first commercialized PRRS subunit vaccine when it hit the market in Taiwan, 2012. The PRRSFREE vaccine features the stimulation of pig's own immune system to defend itself against the virus. By using a detoxified exotoxin of Pseudomonas as a carrier, important genetic information from the open reading frames (ORF) of viruses PRRSV-1 & PRRSV-2 are delivered into the pig's body. ORF1b (essential enzymes for virus RNA synthesis), ORF5 & ORF6 (PRRS envelope proteins, these allow pigs to generate neutralizing antibodies), and ORF7 (proteins that can stimulate immunization) are combined into the little package and directly delivered to the dendritic cells, critical cells that can generate a stronger immune reaction to strengthen defenses and create a long lasting immune memory.

Field tests in Taiwan indicates inoculation at 5 & 8 weeks prior delivery can elevate successful delivery rate by 25% and increase total piglets by 1.5 per litter. Reber Genetics is currently focusing on using the technology of PRRSFREE to create subunit vaccines for other indications such as PCV or CSF. Aiming to develop 3 in 1 (PRRS+PCV+CSF) or multiple indication subunit vaccines in the future.

瑞寶基因在動物疫苗市場上交出亮眼的成績單，然而一路走來並不容易

瑞寶基因的章修綱董事長提到，1988年創辦的瑞安大藥廠是個很重要的起點，不僅對國際新藥研發法規及臨床實務有更多了解，也建立標準化流程，為之後成立的生寶臍帶血、瑞寶基因、生控基因、及安基生技奠下良好基礎。2003年爆發SARS疫情，生寶開始投入免疫相關領域的研發，爾後與農業科技研究院的動物科技研究所合作，將「對標靶細胞專一的融合抗原」及「逆向基因工程技術」兩項專利技術平台技轉至瑞寶基因，進而創造更能提升豬隻對PRRSV免疫力的次單位疫苗，是目前全球唯一研發PRRSV次單位疫苗成功的企業。

瑞寶基因 章修綱董事長

提到該技術平台的特色，章修綱董事長表示：「我們是全球首先利用標靶免疫細胞傳輸系統發展出之PRRS次單位疫苗，也是全世界第一支藍耳病的次單位疫苗。傳統疫苗是在體內產生抗體以預防外來病毒，但若病毒早已存在體內或組織內則不見得有效；而此平台是啟動T細胞免疫反應攻擊病毒，和傳統的活病毒疫苗相比，沒有病毒重組的風險、亦沒有活病毒疫苗造成的初期免疫抑制。該技術也擁有台灣、美國、中國、韓國、澳洲、與歐盟之完整專利保護。」瑞寶基因事業開發部經理張家榮博士則補充，PRRSV病毒株大致可分為歐洲株和美洲株，目前台灣較常使用的美洲株病毒疫苗對歐洲株並沒有抵抗力；而瑞寶PRRSFREE®疫苗是由蛋白質次單位組成，不含病毒，故相較國際大廠生產之PRRS活病毒疫苗不僅安全性較高，對於歐洲株、美洲株、甚至中國及東南亞的高致病性病原株也都具有保護力。瑞寶基因亦可透過基因定序技術快速取得病毒基因序列，最快可在不到三個月的時間內設計並量產出對抗新的突變病毒株的疫苗，較傳統疫苗開發快上許多，是對付爆發性流行傳染病的一大利器。

The Rocky Road to Reber Genetics' Major Success in the Animal Vaccine Industry

Hsiu-Kang Chang, chairmen of Reber Genetics, mentioned that the foundation of Purzer Pharmaceutical was an important beginning. It established understanding in new drug development regulations and solid experience in conducting clinical trials. It also created the SOP that laid foundation for future companies like HealthBanks Biotech, Reber Genetics, Vax Genetics and Annji Pharmaceuticals. In 2003, when SARS erupted in Taiwan, HealthBanks Biotech began to invest in immunological research. Later cooperating with Animal Technology Institute of the Agriculture Technology Research Institute, incorporating exclusive "target cell fusion antigens" and "reverse genetic engineering" technology into Reber Genetics, thus the birth of the globes first commercially effective PRRS subunit vaccine. When speaking of its technology, chairman Chang stated: "we're the world's leading team to utilize the immune cell target transportation system to develop a PRRS vaccine. Traditional vaccines stimulate antibody production within the host's body, but if the pig is already infected prior vaccination, then such methods has its limitations. Our technology directly activates T cells to attack the virus, no recombination risks, no initial immune-suppression. This technology is also well protected with patent rights in Taiwan, the United States, China, Korea, Australia and the Europe Union." Dr. Chang, manager of business development further explained the PRRS virus is roughly categorized into the North American strain and the European strain. Taiwan mostly adopts the North American strain vaccine and is therefore quite defenseless against the European strain. But Reber Genetics' PRRSFREE® vaccine is a subunit vaccine, with no risks of containing live virus and has cross protection over the European and North American strains, even extending its protection to more local China or South East Asia viruses. Reber Genetics can also acquire the genome sequence of a mutated pathogen through latest sequencing technology, and redesign a new vaccine within three months. This is a lot swifter than traditional vaccine development, and can be powerful when facing with newly emerged epidemics.

策略調整：從競爭到雙贏的kingmaker概念

瑞寶技術除了獲得台灣農民青睞，也已在俄羅斯、菲律賓、韓國、泰國等地取得藥證與訂單。關於營銷地點的選擇，章董事長表示：「我們將全球劃分為三個區塊：其一是中國，可說是個獨立的市場；其次為歐美、加拿大、澳洲，中歐美加澳我們積極的尋找策略夥伴合作；其三則是亞洲，如日韓、俄羅斯、東南亞一帶，目前屬於瑞寶原廠銷售。」然而在去年，瑞寶基因做了策略上的調整，從與其他疫苗廠商的競爭關係，轉換為「造王(kingmaker)」的概念，致力將與瑞寶合作的企業推向世界第一邁進，主打化敵為友，尋求雙贏。在各大國際會議及今年六月的第25屆國際豬病大會(International Pig Veterinary Society Congress, IPVS 2018)尋找合適的策略夥伴，未來希望拓展到牛、羊、貓、狗等其他哺乳類動物，甚至禽類的疫苗。

Change of Route: From Competition to Win-win Cooperation

Reber Genetics has earned the trust of farmers in Taiwan, and had also gained drug license and orders from Russia, the Philippines, Korea and Thailand. Chairman Chang also spoke of the choice of target markets. "We've divided the world into three main regions: China itself basically is an independent market. Then there is the European, North American, and Australia regions, where we are still actively looking for partners. And the third would be the remaining countries in Asia, such as Japan, Korea, Russia and ASEAN countries where Reber Genetics is currently focusing on." However, a few adjustments were made to their strategy last year. Instead of competing with other companies, Reber Genetics is seeking a more cooperative route. Adopting the "kingmaker" concept, Reber Genetics seeks partnership with its core technology as its bargaining chip, hoping to reach industrial peak through making alliance with other companies, creating a win-win solution. They will be seeking strategic partners through international conferences, like the upcoming 25th International Pig Veterinary Society Congress (IPVS) in June 2018. In the future, Reber Genetics expects their core technology can be utilized in vaccines of other animals as well, such as cattle, sheep, cat, dogs or even poultry.

專利申請放眼全球，呼籲相關單位多加支持

對於生技人相當關心的專利申請，張博士表示，瑞寶的技術平台獨步全球且布局完善，目前沒有其他競爭者，在專利申請上並不困難，反而是在產學合作的經驗中，發現學界在專利申請上，通常只注意到台灣及美國的專利，而缺乏歐洲及中國的專利申請，因此「全球化」的概念相當重要，除了專利寫作，專利涵蓋的範圍也是關鍵。最後，張博士說：「其實，台灣農業生技有很好的技術涵量，若只評估台灣市場，產值容易被低估，較不受重視;但若評估全球市場，台灣農業生技的產值是相當可觀的，希望官學界及大眾上能多給予支持，幫助台灣的農業生技行銷全世界。」

Global Patent Coverage & Raising Awareness

As for patent protection that is immensely crucial, Dr. Chang assured Reber Gene's strategy is complete and exclusive. Currently, their technology has no immediate competition thus patent application is not as hard as imagined. However, through past cooperation experience with the academy, Dr. Chang noticed that the academy usually narrows its focus on gaining patents solely in the United States and in Taiwan, neglecting China and other European countries. To apply globally according to the target markets is as critical as what the content the patent covers. On a last note, Dr. Chang stated, "Taiwan's agri-biotech has great potential, but if you only assess the market value you're likely to underestimate what we're capable of. We hope to raise awareness among the government, academy and public to help launch Taiwan's agriculture to the global market!"

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