

# 羊痘減毒活毒及次單位疫苗研發結果

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## 摘要

羊痘係羊隻的一種高度傳染性病毒性疾病，以皮膚發紅疹 (erythematous eruptions) 為主要特徵。病程初期，病變呈現丘疹 (popular)，隨後轉為膿疱疹 (pustular eruptions)，至全身性病變時，可能會有呼吸道和胃腸道黏膜出血性炎症反應發生並造成死亡。羊痘病毒，屬痘病毒科中之羊痘病毒屬 (Capripoxvirus)，只有一種血清型。在自然情況下，綿羊痘和山羊痘具有宿主特異性，彼此間具有免疫抗原相關性。病毒顆粒大小為 290 x 270 nm，病毒表面具有管狀小體構成絲狀結構之痘病毒。病毒核酸 DNA 長約 150 Kbp。

自台灣野外分離之羊痘病毒經以山羊睪丸細胞 (Goat Testis cell, GT) 馴化 72 代之本土羊痘病毒已完成疫苗安全及效力試驗，馴化之羊痘病毒分別以 100 及 10 劑量經腋下皮下接種方式進行疫苗安全試驗，結果 100 劑量接種羊隻於接種後 5 至 7 日體溫呈現 40.0 至 40.5 °C 之輕微發燒，但於接種部位或尾根部及腹部均未引發發痘病變。此結果顯示以 GT 馴化 72 代之 R1520 病毒株毒力已明顯下降。此外 1 劑量免疫羊隻均可以耐過羊痘野外強毒攻毒，羊隻健存且未引發皮膚病變。本年度已完成 P32 次單位疫苗佐劑測試，將表現之重組 P32 經過適當的 re-folding 後，分別與 IMS1313、ISA70、QuilA 等佐劑做適當的混合與乳化後免疫 Balb/c 小鼠。所有的小鼠在經過 P32 的兩次免疫後皆有抗體陽轉的情形，尤其是經 ISA70 佐劑所製備的疫苗組，在一次免疫即可達明顯的抗體揚升。不同 pH 值所收得之 re-folded P32 在 ELISA 評估上皆有相似的抗原性。上述 P32 次單位疫苗以每劑量 20 及 60 µg 羊痘 P32 次單位疫苗免疫之羊隻均可耐過羊痘野外強毒攻毒，健存且未引發羊隻全身性皮膚病變，結果顯示試製之羊痘 P32 次單位疫苗具高度安全性及保護力。

# **Development Results of Live Attenuated and Subunit Goat Pox Vaccines**

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## **Abstract**

Goat pox is a highly contagious viral disease, characterized as developing erythematous eruptions. At the preliminary stage of this disease, the goat appeared popular lesions, and followed by pustular eruptions. Possible death caused by hemorrhagic inflammation in the mucosa of respiratory and gastrointestinal tract happened if systemic infection occurred. Goat pox virus belongs to *Capripoxvirus* genus of the *Poxviridae* family, and only one serotype exists within this virus. In the most ordinary situations, sheep pox virus (SPV) and goat pox virus (GPV) perform virus-specificity but related antigenicity exists between these two virus. GPV is about 290 x 270 nm and covered in short tubular filaments on the surface. Genome of GPV is composed of DNA in 150 Kbp.

Attenuated GPV derived from local Taiwan isolate has been experienced 72 passages in GT cells, which has as well been conducted safety study by inoculating 100 or 10 doses per goat subcutaneously into armpit area. The goat accepting 100 dose showed slight elevation in body temperature of 40.0 to 40.5 on day 5 to 7 after inoculation, but no papule lesions appeared at the inoculation sites, root of tail, or abdomen part. This result indicated R1520 experiencing 72 passages in GT performed obvious decline in virulence. The goat proved Safety-certificated of 1 dose all could be protected from wildtype virus challenge without any skin lesions. Adjuvant selection for P32 subunit vaccine has been completed in this year by immunizing Balb/c mice with the purified and re-folded P32 mixed well with adjuvants of IMS1313, ISA70, or QuilA respectively, and all mice exhibited sero-conversion based on the P32 ELISA 2 weeks after one booster, especially in the group accepting P32-ISA70 performing obvious titer evaluation after one shot. The re-folded P32 harvested from different pH value all showed similar antigenicity based on the ELISA evaluation. Goat accepting the above P32 subunit vaccine of 20 or 60µg per dose all could be protected from the wildtype virus challenge without any skin lesions, and it means the prepared P32 subunit vaccine displays highly safety and effect.