• 系統編號	RW9703-2114
• 計畫中文名稱	利用免疫蛋白体學尋找兔化豬瘟疫苗株與野外毒力株抗原差異之研究
• 計畫英文名稱	Development of Immunoproteomics Platforms to Identify Candidate Antigens of Classical Swine Fever Virus for Discriminating Diagnosis
• 主管機關	行政院農業委員會
• 計畫編號	96 農科-14.2.1-檢-B9(4)
• 執行機構	台北醫學大學醫學檢驗暨生物技術學系(所)
• 本期期間	9608 ~ 9612
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• 使用語言	中文
• 研究人員	梁有志 Yu-Chih Liang
• 中文關鍵字	豬瘟病毒；蛋白體學；檢驗試劑
• 英文關鍵字	Classical Swine Fever Virus；Proteomic；Diagnosis Kit
• 中文摘要	防治豬瘟疾病，都是經由注射兔化豬瘟疫苗，達到預防豬瘟的發生與擴散。然而在不注射疫苗的撲滅病毒政策下(non-vaccination stamping-out policy)，在不久的將來，勢必做到不注射豬瘟疫苗，而卻不發生疫情，在這過程中冒然中斷注射疫苗，風險非常的大。所以首先需區別感染豬與接種豬，找出感染豬並撲滅之，即能逐步消滅豬瘟病毒。在這個計畫中我們試圖以免疫蛋白體學(Immunoproteomic)相關技術，尋找兔化豬瘟疫苗和野外病毒感染，是否會引起豬隻產生不同的抗體反應，若能找到差異性的抗原，即可做成檢驗試劑，區別感染豬與接種豬。首先我們從病毒感染的 PK15 細胞，收集 LPC 及野生 CSFV 病毒顆粒，再萃取粗蛋白質。其次以二維電泳法層析這兩種病毒蛋白質，以 Sypro Ruby 染色或轉置到 PVDF 薄膜進行西方點墨分析。在二維電泳法層析中，雖然發現野生 CSFV 病毒與 LPC 病毒蛋白質點表現有所不同，然而以野外感染豬與疫苗接種豬的血清，進行西方點墨分析，卻找不到具有區別性的抗體抗原反應，雖然很多的研究利用免疫蛋白體學相關技術，尋找到新的抗原如，塵蟎及結核桿菌。然而合併前一年的計畫，我們都沒有找到區別性的抗原，我們認爲可能有三大原因。第一，是收集到的病毒及野外病毒感染豬血清的樣品不足且批號不夠多；第二，可能是兔化豬瘟疫苗和野外病毒感染所引起的抗體反應是一樣的。第三，兔化豬瘟疫苗和野外病毒感染所引起的抗體反應有些微不同，然利用免疫蛋白體學相關技術不足以呈現之。
• 英文摘要	The prevention of CSF usually uses the vaccine which made from attenuated LPC strain. At present, the non-vaccination stamping-out

policy is in progress. If suddenly disrupt the vaccination, there would arise CFV disease around nation-wide. Before stop of vaccination, how to differ the pigs between vaccinated and infected is a very important. In this plan, we want to discriminate between LPC-vaccinated and wild CSFV-infected pigs by immunoproteomic technique. First, we harvested the virus particles from PK15 cells cultured medium and then collected the total protein lysate both of LPC and wild CSFV. Second, the protein lysate were separated by two-dimensional electrophoresis (2-DE), and gain the 2-DE maps of the LPC strain and wild CSFV strain by Sypro Ruby staining. Third, 2-DE membranes of LPC strain and wild CSFV strain were immunoblotted with pooled sera from LPC-vaccinated pig or wild CSFV-infected pig. Athough there are several different proteins between the LPC and wild CSFV strains in the 2-DE maps, it seems have no any different spots by immunoblotting assay. Many studies successfully discovery the new antigens by immunoproteomic technique, such as Dust mite and Mycobecterium Tuberculosis. However, we unable to find the new antigen for discriminate between LPC-vaccinated and wild CSFV-infected pigs by immunoproteomic technique. It is possible that 1). the number or amounts of virus samples is not enough to analyze in 2-D system; 2) they produced same antibody pattern; 3) they may have a litter of difference in the antibody pattern, but can not detect by immunoproteomic technique.