

赴泰國評估動物疾病診斷試劑並研習豬瘟免疫學相關技術之出國報告

豬瘟研究組

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摘 要

我們在泰國農業合作部畜產發展處 (The Department of Livestock Development, Ministry of Agriculture and Cooperatives) 的安排下，於本 (98) 年7月19日至24日赴泰國國家動物衛生研究所 (National Institute of Animal Health; NIAH) 及東南亞口蹄疫區域參考實驗室 (Regional Reference Laboratory for Foot and Mouth Disease in South East Asia; RRL) 進行台泰農業合作決議事項之實驗室技術交流，執行有關「口蹄疫病毒非結構蛋白免疫色層分析測試片及豬與禽流感病毒 DNA 晶片診斷試劑」之測試評估。有關口蹄疫非結構蛋白抗體檢測，從泰國提供的 281 個豬隻血清檢體測試結果顯示，口蹄疫非結構蛋白 (NSP) 抗體免疫色層分析測試片之確效試驗 (validation)，檢測經口蹄疫 Asia 1 病毒株攻毒後第 14 天之豬隻血清的診斷敏感性可達 93.8% (15/16)，且與 VIA-AGID 及 UBI 診斷試劑套組檢測結果完全符合，因此證實本測試片也可檢測口蹄疫 Asia 1 血清型病毒感染血清之 NPS 抗體。另以免疫口蹄疫三價疫苗 (含 O, A, Asia 1 血清型) 之豬隻免疫血清及陰性血清檢測結果，其診斷特異性分別可達 98.1% (155/158) 及 100% (31/31)，且與 PrioCHECK FMDV-NS 診斷試劑套組檢測結果之符合率達 98.6% (142/144)。本所開發豬及家禽流行性感冒病毒 DNA 晶片抗原檢測原型試劑亦攜帶到國家動物衛生研究所進行評估測試。該所共提供 17 個流感病毒及 8 個新城病病毒檢體，經 DNA 晶片測試結果顯示，流感病毒可同步區別流感病毒 H1、H3、H5、H6、H7 及 H9 亞型，並可鑑別 H5N1 高病原性家禽流行性感冒病毒株，測試結果與預期相符。此外，豬瘟為國內極重要之豬隻病毒性傳染性疾病，已有報告指出細胞性免疫對於豬瘟的免疫成效影響很大，為了吸取與建立豬瘟特異性細胞性免疫反應之檢測技術，此行亦前往泰國朱拉隆功大學 (Chulalongkorn University) 研習有關應用 ELISPOT 技術檢測豬瘟特異性細胞免疫反應與應用流式細胞儀檢測豬瘟病毒特異性干擾素 (interferon-gamma) 等檢測技術。

Taiwan-Thailand Agricultural Cooperation: Evaluation of Diagnostic Kits and Immunological Study on Classical Swine Fever

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Abstract

We visited the National Institute of Animal Health (NIAH) and the Regional Reference Laboratory for foot and mouth disease in South East Asia (RRL) for evaluating FMDV non-structural protein (NSP) chromatographic strip and SIV-AIV DNA chip and for study of cell-mediated immunity in CSFV infected pig. Under the Taiwan-Thailand Agricultural Cooperation Project, the technical visit, during July 19th to 24th of 2009, was organized by the Department of Livestock Development, Ministry of Agriculture and Cooperatives, Thailand. To evaluate the chromatographic strip assay for detecting anti-NSP antibodies, 281 pig sera provided by the RRL was tested. Diagnostic sensitivity of the assay in FMDV Asia 1 experimentally infected pigs was 93.8 % (15 / 16), completely agreed with the results obtained from the VIA-AGID and UBI ELISA kits. The results revealed that the assay was able to detect FMDV NSP antibodies elicited by the infection of FMDV serotype Asia1. Sera from pigs vaccinated with trivalent inactivated FMDV vaccine (serotype O, A and Asia 1) and naïve pigs were also used to assess the specificity of the assay. The specificity was 98.1 % (155 / 158) for vaccinated pigs and 100 % (31 / 31) for naïve pigs. Agreement between the results from strip assay and those from the PrioCHECK FMDV-NS kit was 98.6 % (142 / 144). To evaluate the prototype of DNA chip for detecting and genotyping swine and avian influenza viruses, 17 influenza viruses and 8 Newcastle disease viruses provided by the NIAH were tested. The DNA chip demonstrated its ability to identify H1, H3, H5, H6, H7, and H9 subtypes of influenza viruses of either swine or poultry origin. Moreover, H5N1 subtype, a highly pathogenic avian influenza virus, can be simultaneously identified as well. For studying cell-mediated immunity (CMI) in pigs induced by classical swine fever virus (CSFV), we met Dr. Sanipa Suradhat at Department of Veterinary Microbiology, Faculty of Veterinary Science, Chulalongkorn University and worked with her colleagues. Dr. Suradhat presented their achievements and the technology that has been set up in her lab for monitoring CSFV-CMI in pigs. Through this visit, we also learned how to detect CSFV-specific interferon-gamma secreted by peripheral blood mononuclear cells in pigs using ELISPOT or flow cytometry.