非洲豬瘟抗體ELISA檢測技術之研發

豬瘟研究組

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摘要

以原核系統表現非洲豬瘟病毒P72及P30結構蛋白，表現之重組蛋白經Ni-NTA管柱純化後顯示P72及P30皆有產物表現，且為可溶性蛋白。以Western blot測試顯示，2種重組蛋白皆可辨識非洲豬瘟陽性豬血清。以ELISA法測試，P72重組蛋白對非洲豬瘟陽性豬血清之抗原抗體反應效果較P30為佳。P72重組蛋白經純化後披覆於微量免疫盤建立非洲豬瘟抗體ELISA檢測方法，以台灣豬場收集1,163件非洲豬瘟陰性血清測試自製間接型ELISA套組之反應效能，並以進口非洲豬瘟陽性豬血清做為陽性對照。結果顯示，以陰性血清平均值加3個標準偏差(3SD)做為臨界值(Cut-off)，自製間接型抗體ELISA檢測之特異性為 97.5%。以商品化非洲豬瘟阻斷型(Blocking)ELISA套組測試國內豬場255件非洲豬瘟陰性豬血清，結果顯示，檢測之特異性為100%。

Development of ELISA for detection of antibody to African swine fever virus

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Abstract

The P72 and P30 structural proteins of African swine fever virus (ASFV) were expressed in the prokaryotic system. The expressed recombinant proteins were purified by Ni-NTA column, and each protein was expressed at MW of 72 and 30 kDa, respectively, and they were all soluble proteins. The Western blot test showed that the two recombinant proteins can be recognized by positive pig serum from African swine fever virus infection. Another tested by the ELISA method, the P72 recombinant protein showed better antigen-antibody reactivity to ASFV-positive serum than P30. The P72 recombinant protein was purified and coated to the microplate to develop the indirect-ELISA method for African swine fever antibody detection. 1,163 African swine fever negative sera collected from Taiwan pig farms were used to test the efficiency of the homemade indirect-ELISA kit, and the imported ASFV-positive serum was selected as a control serum. The results showed that taking the mean of negative serum plus 3 folds of standard deviations (3SD) as the cut-off value (Cut-off), the specificity of homemade indirect antibody-ELISA was 97.5%. A commercial African swine fever blocking ELISA kit was used to test 255 African swine fever negative swine sera from domestic pig farms. The results showed that the specificity of the detection was 100%.