口蹄疫病毒非結構性蛋白抗體檢測 ELISA 之田間應用

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摘要

口蹄疫 (FMD) 為豬隻高度傳染水泡性疾病，常造成養豬業者之為害。本試驗主要利用原核表現系統完成建立口蹄疫病毒株(FMDV)非結構性蛋白(FMDV-NSP) Sandwich ELISA抗體檢測區別診斷方法。主要著重在FMDV基因組上NSP免疫決定位之核酸序列設計特異性引子，以RT-PCR方法增幅病毒基因，經由原核選殖表現系統進行3ABC、3A、3B、2C及3D等基因，經試驗結果確認五種FMDV-NSP核酸序列定序結果皆符合，且構築表現重組蛋白質分子量如同預期大小。於FMDV Sandwich ELISA之NSP抗體檢測分析敏感性結果證實極限稀釋達1:100，且可與牛、豬及羊感染FMDV抗血清呈專一特異性結合反應。另以Sandwich ELISA NSP抗體檢測174支田間豬隻血清樣品比較商品化ELISA試劑套組結果符合率大致相同。最後證實本試驗方法不會與豬水疱病病毒(SVDV)及矽尼卡谷病毒(SVV)等誘發的抗體產生非特異性反應。

Detection of Antibodies against Non-Structural Proteins of Foot-and-Mouth Disease Virus using ELISA: a field application

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Abstract

Foot-and-mouth disease (FMD) is a highly contagious blistering disease in pigs that causes serious economic losses on pig farms. In this project we use a prokaryotic expression system to establish a double antibody sandwich ELISA diagnostic method for the detection of antigens of non-structural proteins (NSP) of the foot-and-mouth disease virus (FMDV). The first tasks were to design specific primers that bind to NSP immunological epitopes on the FMDV genome, and to amplify the target genes using RT-PCR. The 3ABC, 3A, 3B, 2C and 3D genes were then cloned and expressed through the prokaryotic expression system. The results showed that the sequencing of the five FMDV-NSP nucleic acid sequences were in frame, and the molecular weight of the expressed recombinant proteins were determined as predicted. The sensitivity of the FMDV-NSP antibody assay using the sandwich ELISA method confirmed that the limiting dilution was 1:100, and that it could specifically bind to the FMDV antisera in cows, pigs and goats. The results obtained from the sandwich ELISA were consistent with those from a commercial ELISA kit in detecting sera from 174 pigs in the field. Neither antisera against swine vesicular disease (SVDV) nor sera against Seneca valley virus (SVV) could be detected by this sandwich ELISA method.