豬水疱病抗體 ELISA 檢測方法之研發

豬瘟研究組

潘居祥 副研究員

摘要

豬水疱病 (SVD) 是豬的傳染性病毒性疾病，會造成冠狀帶及蹄趾間的水疱，有時在唇、舌、鼻吻及乳頭也有水疱病變。豬水疱病病毒 (SVDV) 被分類在豬腸病毒族群，屬於小核糖核酸病毒科，其抗原性與人類 Coxsackie B5 型病毒有相關，會造成血清交插反應。本病屬於須通報的動物傳染病，歐洲各國皆以撲殺及嚴格限制牲畜移動方式來控制本病。根據 OIE 陸生動物衛生法典所載，抗體檢測是診斷本病的重要方法。本試驗發展 SVD 抗體 ELISA 檢測方法，以 RT-PCR 方式增幅 SVDV 之 VP1 及 VP2 全長基因並加入限制酶切位。將 RT-PCR 產物選殖入 TOPO 載體進行 TA 選殖 (T-A cloning) 並定序確認序列正確。再將標的基因分別次選殖入 pET28、pET32、SUMO、pCold 等四種不同表現載體，經定序確認之質體分別轉形至 BL-21(DE3) 菌株，以 IPTG 誘導後測試表現重組蛋白之溶解性，結果顯示，僅 pCold 載體表現之重組蛋白呈現水溶性。以 western blot 測試顯示重組蛋白可辨識 SVD 陽性豬血清，且 VP1 重組蛋白抗原性高於 VP2 蛋白。將表現之水溶性重組蛋白以市售不同廠牌 His taq 蛋白純化套組及切膠純化方式比較純化後重組蛋白之純度，結果顯示，以切膠純化方式所得之純度最高。將純化之 VP1 重組蛋白以適當濃度披覆於 ELISA 抗原盤，以 Indirect ELISA 方式測試連續稀釋 之 SVD 陽性豬血清，可得到線性曲線。

Development of an indirect ELISA for the detection of antibodies to swine vesicular disease virus.

Chu-Hsiang Pan

Abstract

Swine vesicular disease (SVD) is a contagious disease of pigs, characterized by vesicles on the coronary bands, heels of the feet and occasionally on the lips, tongue, snout and teats. Swine vesicular disease virus (SVDV) is a member of the genus Enterovirus and family Picornaviridae. The virus is antigenically related to the human coxsackievirus B5. SVD is a notifiable disease and is strictly controlled in European countries by stamping out and restriction of pig movements. According to the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, SVD antibody detection is a prescribed test for international trade. In this study, we developed an enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies to swine vesicular disease virus. Full length of VP1 and VP2 genes of SVDV were amplified by RT-PCR and the restriction enzyme cutting sites of BamHI and HindIII were added in the both ends of primer site. The RT-PCR amplicons were cloned into TOPO vector by a TA cloning kit and then the sequences were confirmed by DNA sequencing techniques. The target genes were subclone into the pET28, pET32, SUMO and pCold expression vector individually. The expression plasmids were transformed into the BL-21(DE3) host cell. IPTG is used to induce expression of cloned genes and the solubility of recombinant proteins was tested. Results showed that only pCold vector expressing products belonged to soluble protein. His taq protein purification method and gel extraction method were used to compare the purity of recombinant proteins. Results showed that gel extraction method could obtain recombinant protein in high purity. The purified recombinant protein of VP1 was coated in ELISA plates and indirect ELISA method were used to test a serial dilution of pig serum which obtained from the experimental SVDV infected pig. Results showed that linear curve could be observed in the indirect ELISA assay.