區別偵測豬環狀病毒第二型a及第二型b的殼鞘蛋白質

豬瘟研究組
洪鈴柱 助理研究員

摘要

豬環狀病毒第二型(PCV2)為最小且含單股環狀去氧核醣核酸基因組的無封套病毒，該病毒常造成養豬產業的重大經濟損失。目前，已確認PCV2有兩種主要的基因型：PCV2a和PCV2b。通常患有豬環狀病毒第二型相關疾病（PCVD）的豬隻較常分離出 PCV2b，據文獻指出PCV2b比PCV2a更具毒力。本研究的目的是生產不同特殊抗體，分別抗PCV2開放閱讀框二(ORF2)蛋白質(又稱殼鞘蛋白質)的能力，並利用這些抗體開發抗原捕獲酵素聯結免疫吸附法套組(AC-ELISA)去區別偵測豬環狀病毒第二型a及第二型b的殼鞘蛋白質。至此分別生產不同抗PCV2a和PCV2b的單株抗體。利用克隆型別(ClonotypingTM)系統區別單株抗體同位型別。以西方墨漬法試驗分析單株抗體與殼鞘蛋白質的特異結合。某些單株抗體在蛋白質分子量27 kDa及30 kDa有特異性條帶反應；某些單株抗體在蛋白質分子量27 kDa、30 kDa及60 kDa均有特異性條帶反應。進一步發現，某些單株抗體可以在PCV2感染豬隻的淋巴球及豬環狀病毒螢光分析受質樣本的玻片均有陽性反應。利用不同單株抗體及捕捉抗體組成AC-ELISA，並檢測PCV2a組成的疫苗樣本、PCV2b(ORF2)重組蛋白質及豬隻全血的樣本。結果顯示自製檢測套組可區別偵測PCV2a及PCV2b的殼鞘蛋白質。

Differential recognition of capsid proteins from PCV2a and PCV2b

Ling-Chu Hung,
Division of Hog Cholera Research

Abstract

Porcine circovirus 2 (PCV2) is a small, non-enveloped virus with a single-stranded circular DNA genome that has had severe impacts within the hog industry. Currently, two major genotypes of PCV2 have been recognized, PCV2a and PCV2b. PCV2b has been more frequently isolated from pigs with PCV-associated diseases (PCVAD) and may be a more virulent genotype than PCV2a. The purpose of this study was to generate antibodies anti ORF2 protein (capsid protein) of PCV2 and developing an antigen-capture enzyme-linked immunosorbent assay (AC-ELISA) kits which can detect capsid proteins from PCV2a or PCV2b. Therefore, I generated couples of monoclonal antibodies (mAbs) for PCV2a and PCV2b, respectively. The isotype of the produced mAbs was determined using a ClonotypingTM System. The reactivity of the mAbs to PCV2 capsid protein was determined in a Western blot assay. Some mAb gave a strong and specific reaction with proteins of approximately 27 kDa and 30 kDa. For some mAb, faint but specific bands were observed at 27 kDa, 30 kDa, and 60 kDa respectively. Furthermore, the specificity of mAbs showed positive signals on PCV2 infected swine lymphocytes and in Porcine Circovirus FA substrate slides (VMRD, USA) by indirect immunofluorescence staining. Moreover, we were able to detect different PCV2 capsid proteins with AC-ELISA in PCV2a based vaccine samples, PCV2b ORF2 recombinant protein, and pig whole blood samples. This study demonstrates that these home-made kits can detect capsid proteins from PCV2a or PCV2b.