

應用即時定量 RT-PCR 同時檢測豬隻糞便檢體中 Sapelovirus 及 PEV B 病毒核酸

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摘要

豬薩佩羅病毒與 B 型腸病毒是引起豬隻神經系統、呼吸系統、繁殖系統以及皮膚系統等多種障礙之主要病原之一，若一旦與其他豬隻病原如：豬瘟病毒（CSFV）、豬生殖與呼吸綜合症病毒（PRRSV）以及豬小病毒（PPV）等病原混合感染將造成患豬嚴重的病徵。為了解台灣田間豬隻薩佩羅病毒與 B 型腸病毒感染情形，我們嘗試應用即時定量反轉錄聚合酵素連鎖反應(real time RT-PCR)開發可同時偵測並鑑別兩種不同病毒核酸之方法。目前的結果顯示，我們所設計針對兩種病毒基因之探針與引子對可同時進行 Multiplex 反應而不會互相干擾，其中針對豬薩佩羅病毒之偵測敏感度約為 $10^{2.23}/\text{ml TCID}_{50}$ ，而 B 型腸病毒敏感度亦相同。因此，應用即時定量 RT-PCR 建立快速、準確並可同時區分 Sapelovirus 及 PEVB 病毒核酸之分子病毒學診斷方法，在分析豬隻糞便檢體中 Sapelovirus 及 PEVB 上將可有效縮短病毒分離及傳統 PCR 所耗費之時間。

Application of real time RT-PCR to identify the RNA of Sapelovirus and PEV B in swine fecal swab

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Abstract

Porcine sapelovirus and enterovirus B (PEV B) are the major pathogens responsible for neurological disorders (such as encephalomyelitis), respiratory, reproductive and dermal lesions in swine. Recent studies have reported that co-infection of PEVB and other pathogenic virus such as classical swine fever virus (CSFV), porcine reproductive and respiratory syndrome virus (PRRSV) and porcine parvovirus (PPV) usually results in severe syndromes of swine diseases in infected pigs. To investigate the infective situation of porcine sapelovirus and porcine enterovirus B in swine in Taiwan, we applied the real-time RT-PCR method to detect and differentiate the viral nucleic acid of Sapelovirus from that of PEV B. The results showed that the primers and probes exclusively designed for the detection of these two viruses could be simultaneously used in the multiplex reaction without significant interference. The sensitivity of this reaction for porcine sapelovirus and PEV B could achieve a TCID₅₀ of approximately $10^{2.23}$ /ml. Therefore, the application of real-time RT-PCR can be used as the platform for rapid, accurate, and differential identification of the viral nucleic acids of Sapelovirus and PEV B. It will be more efficient and less time consuming for the virus isolation and identification of Sapelovirus and PEV B from pig fecal samples as well as for the using of conventional PCR method.