不同基因型口蹄疫病毒區別診斷技術之建立

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摘要

口蹄疫(Foot and mouth disease; FMD)屬於高度傳染性病毒性疾病，具感受性之偶蹄動物感染本病常會造成巨大之經濟損失，口蹄疫病毒(Foot and mouth disease virus; FMDV)是小核糖核酸病毒科(Picornaviridae)中口瘡病毒屬(Aphthovirus)的成員，口蹄疫病毒可分為O、A、C、Asia-1、SAT1、SAT2、SAT3等7種血清型，各種血清型別間無交叉免疫保護效果。目前7個血清型中已超過70個基因型被確認，一般送檢病例檢測上，除非採用 DNA microarray 或 DNA 核酸定序方法，否則很難區別口蹄疫病毒不同基因型。為縮短送檢口蹄疫病例檢測時間及確診基因型別，本試驗發展即時定量 RT-PCR 方法。配合 TaqMan 探針技術可將口蹄疫 O 型病毒基因型快速區別，且能進一步區別目前台灣流行的口蹄疫野外毒與目前使用的疫苗毒。總共有5條分別針對口蹄疫病毒 O1/Campos、O1/Manisa、O/Taiwan/99、O/Taiwan/97 與 O/Taiwan/2009 之特異性 TaqMan 探針及一對口蹄疫病毒通用型引子(universal primer)被設計及合成，經由即時定量 RT-PCR 方法可快速檢測及區別不同基因型。在敏感性試驗上，我們設計的引子比 OIE 網站提供的口蹄疫病毒檢測引子其敏感度高約 10 倍。我們結果顯示，利用即時定量 RT-PCR 配合 TaqMan 探針即可快速檢測及區別五種基因型口蹄疫病毒。

Differential diagnosis in different genotype of food-and-mouse disease viruses

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Abstract

Foot-and-mouth disease (FMD) is a highly contagious viral disease affecting cloven-hoofed animals that has a great potential for causing severe economic loss. FMD virus (FMDV) is a member of the genus *Apthovirus* in the family of Picornaviridae. There are seven serotypes of FMDV: O, A, C, Asia 1, and South African Territories (SAT) 1, 2, and 3. Infection with any one serotype does not confer immunity against another. Within the serotypes, over 70 subtypes have been identified. It is difficult to differentiate genotypes of FMDV in routine Lab diagnosis unless analysis of nucleic acid DNA sequences is conducted. In this study, we developed a real-time RT-PCR using TaqMan probes for the detection and subtyping of FMD type O viruses. It can also differentiate between wild-type and vaccine strains of FMD virus type O viruses. A total of five TaqMan probes specific to O1/Campos, O1/Manisa, O/Taiwan/99, O/Taiwan/97 and O/Taiwan/2009 FMD viruses and one universal primer were designed. Using real-time RT-PCR, these five FMD viruses can be detected and genotyped using the TaqMan assay. Our results showed that the sensitivity of the primer is around 10 times higher than that of OIE the one lists in the "Manual of Diagnostic Tests and Vaccines for Terrestrial Animals". Therefore, the real-time RT-PCR combined with the TaqMan assay could be used for the rapid genotyping of five FMD type O viruses.