Diagnosis of foot-and-mouth disease by RT-PCR: use of phylogenetic data to evaluate primers for the typing of viral RNA in clinical samples

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Summary. The results of type-specific RT-PCR diagnostic assays on foot-and-mouth disease (FMD) viruses in clinical samples were mapped onto serotype-specific dendrograms representing the degree of nucleotide sequence variation between the FMD virus isolates. This novel approach assisted the selection of suitable PCR primer sets for the diagnosis of FMD virus isolates belonging to different topotypes within each serotype. These interpretations were qualified by using a universal (FMD virus group) specific primer to confirm that FMD virus RNA had been extracted from the samples under investigation. The analyses showed that the design of primer sets for the detection of FMD virus serotypes O, A, Asia 1, SAT 1 and SAT 3 were generally satisfactory, as most virus isolates within the major virus sub-groupings were successfully detected. However, the FMD virus serotype C and SAT 2 specific primers were less efficient as certain virus sub-groups were not detected. This identified the need for additional or alternative primers to improve RT-PCR procedures for more comprehensive detection of divergent virus strains within these serotypes. There were some examples where not all virus isolates from the same outbreak reacted with particular type-specific primers which suggested that either further minor refinements may be necessary in the primer design or that there were shortcomings in the RT-PCR methodology.

Introduction

There are three particular features of foot-and-mouth disease (FMD) which make rapid, sensitive and specific laboratory tests essential for diagnosis. Firstly, it is the most contagious disease of cloven-hoofed animals and hence has the potential for explosive spread in susceptible livestock populations. Secondly,
is acknowledged as the most serious constraint to international trade in livestock and animal products. Thirdly, other viruses produce symptoms which are clinically indistinguishable from FMD, necessitating laboratory investigation for a definitive diagnosis.

A marked improvement in the laboratory diagnosis of FMD has resulted since ELISA methods were introduced in the mid-1980’s [1, 2]; however, there is still scope for improvement in the sensitivity of antigen detection. The polymerase chain reaction (PCR) method theoretically offers the ultimate sensitivity and a great deal of effort has been made in this laboratory [3–5] and elsewhere [6–8] to explore its usefulness for FMD diagnosis. The main finding from these investigations is that although the RT-PCR protocols perform satisfactorily in many instances, they are insufficiently sensitive and often exhibit a narrower spectrum of reactivity compared with the existing procedures of ELISA coupled with virus isolation in cell culture. The conclusions have generally been that the present reverse transcription (RT-)PCR’s are extremely valuable tools to assist current procedures but that they cannot yet replace them.

Various reasons have been postulated to explain these shortcomings and include the design of the primers and their sensitivity, the methodology of the RT-PCR protocol and the efficiency of the RNA extraction. In this study we have examined further the design of existing serotype-specific primers for the RT-PCR by combining the results achieved in a previous study [4] with additional RT-PCR results obtained on more FMD virus strains and correlating them to the corresponding nucleotide sequences within the VP1 (1D) region of the virus genome.

**Materials and methods**

**Virus strains, sample preparation and RT-PCR**

FMD virus strains were selected in relation to the known molecular diversity which exists within each of the seven FMD virus serotypes ([9]; N. J. Knowles and A. R. Samuel, unpublished data). Sample preparation, RNA extraction, reverse transcription and PCR amplification were as described previously [4]. The sequence, serotype specificity, genomic location and nucleotide position of the oligonucleotide primers are shown in Table 1 and are as previously described [10, 11].

**Sequencing of FMD viruses**

Oligonucleotide primers

Oligonucleotide primers with a Cy5 amidite fluorescent dye for use with the ALFexpress™ automated sequencer were purchased from Pharmacia Biotech.

**RT-PCR and sequencing**

Nucleotide sequences were determined by RT-PCR and cycle sequencing by a fmol™ DNA sequencing kit (Promega, UK) which uses the cycle sequencing method described by Murray [12] as adapted by Knowles and Samuel [13]. The RT-PCR utilised the various primer sets as described previously [13, 14]. The cycle sequencing reactions were essentially the same as the manufacturer’s protocol with the following amendments: approximately 80 fmoles of cDNA template was used in the reactions and 1.5 pmoles of Cy5 amidite-labelled primer.