Use of the Reverse Transcription Polymerase Chain Reaction (RT-PCR) for the Rapid Diagnosis of Foot and Mouth Disease in South America

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ABSTRACT

Foot and mouth disease (FMD) is a limiting factor for the economic progress of the animal industry in South America. The presence of the disease results in the imposition of national and international sanitary barriers to animals and animal products, and, most especially, a reduction in the availability of protein from animal origin and in income. Rapid and accurate identification of infected animals, those with either clinical or subclinical disease as well as with persistent infection, is essential for maintaining an efficient eradication programme. The polymerase chain reaction was used to rapidly identify infected animals. With a primer set that corresponds to a conserved region of the 3D sequence of the viral genome, it was possible to amplify, regardless of the serotype, 116 strains of FMD virus, of which 109 were strains collected from outbreaks of FMD throughout South America from 1945 to the most recent outbreaks in 2000/2001. The PCR technique should be of considerable value in facilitating the diagnosis of FMD in South America, where laboratory resources are limited and a rapid response is needed, particularly in areas where national programmes for controlling or eradicating the disease are being implemented.

Keywords: cattle, diagnosis, foot and mouth disease, polymerase chain reaction, RNA polymerase gene, 3D gene

Abbreviations: BHK, baby hamster kidney; ELISA, enzyme-linked immunosorbent assay; FMD, foot and mouth disease; FMDV, foot and mouth disease virus; OPF, oesophageal–pharyngeal fluid; PCR, polymerase chain reaction; RT-PCR, reverse transcription polymerase chain reaction; SVDV, swine vesicular disease virus; TCID<sub>50</sub>, tissue culture infectious dose 50%; VSEV, vesicular exanthema of swine virus; VSV, vesicular stomatitis virus

INTRODUCTION

Foot and mouth disease virus (FMDV) has a worldwide distribution and is of major importance for the animal industry in South America. The disease is particularly a problem for countries where animals and meat contribute significantly to the national economy through export markets. Foot and mouth disease (FMD) is enzootic in many parts of South America. In the last ten years, the internationally recognized disease-
free area has increased and the incidence of FMD has been progressively reduced. At the same time, the number of affected herds and the morbidity rate in various species continue to decline in the regions where the disease is present. However, new outbreaks of type O and A have recently been identified in areas previously recognized as free of FMD without vaccination (Pan American Health Organization, 2001). This re-emerging disease has prejudiced the economies of affected countries by affecting the productivity of their livestock and by restricting their access to international markets. Rapid and accurate diagnosis of FMD is essential for the maintenance of an eradication programme. A suitable and reliable test must be able to detect all types of FMDV circulating in South America, as well as a low number of viral particles.

The reverse transcription polymerase chain reaction (RT-PCR) has been shown to be a useful tool in the diagnosis of FMD (Marquardt et al., 1995), as a part of the viral genome can be detected with a very high sensitivity in less than 24 h in a wide range of samples (Meyer et al., 1991). Although there have been several reports on the use of RT-PCR with a wide range of primers (Reid et al., 1999) targeting different areas of the FMD genome, the ability of these primers to recognize strains of epidemiological importance in South America has not been evaluated.

In this report, we described a RT-PCR that targets the 3D gene of FMDV (viral RNA polymerase), enabling the identification of sequences of FMDV serotypes A, O and C of epidemiologically relevant strains of the virus isolated in South America from the years 1945 to 2001.

MATERIALS AND METHODS

Virus strains and sample preparation

Cell culture-grown isolates of South American strains of FMDV obtained from 1945 to 2001 and maintained in the Pan American Foot and Mouth Disease Center (PANAFTOSA) collection were used to evaluate the primers. FMDV-positive epithelial samples representative of serotypes A, O and C were also used. All the isolates had been stored at −70°C in phosphate-buffered glycerol. Epithelial field samples received during the evaluation of the RT-PCR were collected in Trizol Reagent (Gibco BRL, Sao Pablo, Brazil).

Swine vesicular disease virus (SVDV), vesicular stomatitis virus (VSV) Indiana and New Jersey strains and vesicular exanthema of swine (VESV) were used to test the specificity of the FMDV primers.

Total RNA was extracted with Trizol Reagent (Gibco BRL) according to the manufacturer’s instructions. The pellets of RNA were resuspended in 20–50 μl of RNAse-free sterile water (Sigma-Aldrich, St Louis, MO, USA).

Oligonucleotide primers

The primers for FMDV were based on published sequences. Primers targeted to the FMDV 3D gene were selected with the aid of the Primer 2 computer program