Detection of Foot-and-mouth Disease Serotype O by ELISA Using a Monoclonal Antibody

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An ELISA assay with monoclonal antibody (MELISA) was used to type serotype O of foot-and-mouth disease virus (FMDV). All FMDV serotype O reference strains were positive by MELISA, while other viruses such as FMDV serotypes Asia 1, C, A and classical swine fever virus, swine vesicular disease virus, and porcine reproductive and respiratory syndrome virus remained negative. Further, FMDV serotype O positive samples were able to be detected by MELISA. This assay may be particularly suitable for diagnosis of FMDV serotype O infection in field stations.

Introduction

FOOT-AND-MOUTH DISEASE VIRUS (FMDV) is a member of the genus Aphthovirus of the family Picornaviridae, which is divided into seven serotypes with no cross-protection conferred among the serotypes.⁽¹⁾ FMDV serotypes O, A are widely distributed worldwide, whereas FMDV serotypes SAT 1, SAT 2, SAT 3 are normally restricted to Africa and FMDV serotype Asia 1 to Asia.^(2,3) Due to the aggressive nature of foot-and-mouth disease (FMD), outbreaks usually result in areas with severe economic loss and impact both national and international trade within the livestock and animal product industry.⁽⁴⁻⁷⁾ Given the extreme contagious nature of the causative virus, rapid and accurate diagnosis of any suspected FMD case is of utmost urgency to control this veterinary infection.

Laboratory diagnosis of FMD is made by conventional enzyme-linked immunosorbent assay (ELISA) detection of specific viral antigens and by observation of cytopathogenic effects in cell culture.^(4,8,9) Alternatively, conventional reverse transcriptase polymerase chain reaction (RT-PCR)^(5,10,12-14) and real-time RT-PCR^(6,11,15-17) have been developed to complement primary diagnostic techniques for the FMDV infection. These assays are time-consuming and laborious, and require centralized laboratory facilities and clinical specimen submissions, resulting in the delay of an FMDV diagnosis. Given these problems, a rapid, simple, and practical assay to detect FMDV in animals and their products are therefore required in clinical practice.

In this study, the MELISA assay using mouse monoclonal antibody was developed as a diagnostic method for the typing of FMDV serotype O. Sensitivity and specificity of the

MELISA assay were then evaluated using clinical samples from the FMDV-infected animals; 125 samples were collected for general surveillance (Table 1) as described previously.^(6,7) All samples were also identified by traditional ELISA, respectively. The details of primers and conditions for the traditional ELISA assay for the detection of FMDV have been previously described.⁽⁸⁾ All animals were handled in strict accordance with good animal practice according to the Animal Ethics Procedures and Guidelines of the People's Republic of China, and the study was approved by the Animal Ethics Committee of China.

To check that the ELISA reaction was specific for FMDV serotype O, the reference strains were tested, which included FMDV serotypes O (O/CHA/1999, O/CHA/2009), A (A/CHA/1972, A/CHA/2009), C (C/SU/1958), Asia 1 (Asia 1/JS/2005), and classical swine fever virus (CSFV), swine vesicular disease virus (SVDV), and porcine reproductive and respiratory syndrome virus (PRRSV).

Materials and Methods

IBRS-2 and BHK-21 cells were maintained in Eagle's minimum essential medium (Takara, Dalian, China) with 1.5% 7.5% NaHCO₃ and 10% fetal bovine serum. A monolayer of IBRS-2 and BHK-21 cells was used for virus propagation. The virus strains FMDV and swine vesicular disease virus (SVDV) were grown on IBRS-2 and BHK-21 monolayer cells. BHK-21 cells were grown in MEM medium (Takara, Dalian, China) containing 4% newborn calf serum, and used to replicate FMDV. FMDV and SVDV inactivated reference antigens were used. Antigens were purified from the type O vaccine by centrifugation. The above-mentioned viruses and inactivated

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TABLE 1. COMPARISON OF DETECTION RESULTS FOR TWO ELISA ASSAYS USING 125 SAMPLES

Pathogen	Strain (specimen no.)	Results (positive no./specimen no. tested)	
		MELISA	Traditional ELISA
FMDV	O/CHA/1999 (32)	+(32/32)	+(32/32)
	A/CHA/2009 (22)	-(22/22)	+(22/22)
	Asia 1/JS/2005 (20)	-(20/20)	+(20/20)
	C/UN/1958 (5)	-(5/5)	+(5/5)
CSFV	C2008 (17)	-(17/17)	-(17/17)
SVDV	SVDV01 (10)	-(10/10)	-(10/10)
PRRSV	HPBEDV (10)	-(10/10)	-(10/10)
JEV	JEV2009 (9)	-(9/9)	-(9/9)

+, positive reaction; -, negative reaction.

antigens were used as the ELISA antigens for the experiment. FMDV strain O/CHA/1999 specific MAb 2G11 and the polyclonal antibody rabbit sera against the FMDV type O used in this study were provided by the national FMDV reference laboratory of China (Lanzhou, Gansu Province).

Each microplate (Corning-Costar, Pleasanton, CA) was coated with rabbit polyclonal antibody (10 µg/mL) in 0.1 M carbonate/bicarbonate buffer (pH 9.4) overnight at 4°C. Plates were blocked with 5% skim milk in PBS and washed three times with PBST (containing 0.1% Tween-20, pH 7.4). After washing three times, 50 µL of ten-fold serial dilutions of the purified FMDV (1 µg/mL to 0.1 pg/mL) were added to each microplate and incubated for 1 h at 37°C. The plates were subsequently washed, and then 50 µL MAb 1D11 (1:1000 dilution) was added and incubated for 1 h at 37°C. After three washings, 50 µL goat anti-mouse IgG, which was conjugated with horseradish peroxidase (HRP) (Sigma, Beijing, China), was added and incubated for 1 h at room temperature. After washing three times with PBST, the MELISA reaction was developed by the addition of ophenylenediamine-H₂O₂ for 20 min at 37°C. The reaction was stopped by the addition of 50 µL 1.5 M H₂SO₄, and the microplate was read at OD490 by a plate reader (Bio-Rad, Hercules, CA).

Results and Discussion

Purified FMDV serotype O strain, O/CHA/1999 was used to determine the sensitivities of MELISA and the traditional ELISA. The results indicated that the detection limit of the traditional ELISA for purified viruses was 0.1 ng, while the MELISA was more sensitive than traditional ELISA and could detect 0.04 ng of purified viruses. The traditional ELISA and MELISA could detect a minimum of FMDV-infected sero diluted at 1:5120 and 1:20,480 (w/v, g mL⁻¹), respectively, which indicated that both methods were sensitive to the detection of FMDV serotype O in field samples. The sensitivity of MELISA for purified virus preparation and viruses in infected nasal swabs/extracts was four times greater than that of the traditional ELISA.

To check that the ELISA reaction was specific for FMDV serotype O, the reference strains were tested in this work. The results indicated that the ELISA assay was able to type the FMDV serotype O strain used in this experiment as each showed the positive result. As expected, the other viruses

containing FMDV serotypes Asia 1, C, A and CSFV, SVDV, PRRSV gave a negative result with the ELISA assay.

To assess the applicability of this method, the experiment was performed using clinical samples. The result indicated that 20 positive samples of FMDV serotype Asia 1 were typed by MELISA, but 32 FMDV serotype O, 5 FMDV serotype C, 22 FMDV serotype A, 17 CSFV, 10 SVDV, 10 PRRSV samples gave a negative result in this study.

Taken together, the MELISA assay was specific and sensitive for typing FMDV serotype O in clinical samples from infected pigs. This method not only significantly reduces the diagnosis time but also may offer the potential for wider use in field practice.

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Author Disclosure Statement

The authors declare that they have no competing interests.

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