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ORIGINAL ARTICLE

Development of a multiplex Luminex assay for detecting swine antibodies to structural and nonstructural proteins of foot-and-mouth disease virus in Taiwan

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multiplex Luminex assay;
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structural protein

Background/Purpose(s): Foot-and-mouth disease (FMD) and swine vesicular disease (SVD) are serious vesicular diseases that have devastated swine populations throughout the world. The aim of this study was to develop a multianalyte profiling (xMAP) Luminex assay for the differential detection of antibodies to the FMD virus of structural proteins (SP) and nonstructural proteins (NSP).
Methods: After the xMAP was optimized, it detected antibodies to SP-VP1 and NSP-3ABC of the FMD virus in a single serum sample. These tests were also compared with 3ABC polypeptide blocking enzyme-linked immunosorbent assay (ELISA) and virus neutralization test (VNT) methods for the differential diagnosis and assessment of immune status, respectively.
Results: To detect SP antibodies in 661 sera from infected naïve pigs and vaccinated pigs, the diagnostic sensitivity (DSn) and diagnostic specificity (DSp) of the xMAP were 90.0—98.7% and 93.0—96.5%, respectively. To detect NSP antibodies, the DSn was 90% and the DSp ranged from 93.3% to 99.1%. The xMAP can detect the immune response to SP and NSP as early as 4 days postinfection and 8 days postinfection, respectively. Furthermore, the SP and NSP antibodies in all 15 vaccinated but unprotected pigs were detected by xMAP. A comparison of SP and NSP antibodies detected in the sera of the infected samples indicated that the results from the xMAP had a high positive correlation with results from the VNT and a 3ABC polypeptide blocking ELISA assay. However, simultaneous quantitation detected that xMAP had no relationship with the VNT. Furthermore, the specificity was 93.3—94.9% with 3ABC polypeptide blocking ELISA for the FMDV-NSP antibody.

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Conclusion: The results indicated that xMAP has the potential to detect antibodies to FMDV-SP-VP1 and NSP-3ABC and to distinguish FMDV-infected pigs from pigs infected with the swine vesicular disease virus.

Introduction

Foot-and-mouth disease (FMD), swine vesicular disease (SVD),[1] vesicular stomatitis (VS), and vesicular exanthema of swine are serious vesicular diseases that have devastated swine populations throughout the world. Foot-and-mouth disease is caused by the foot-and-mouth disease virus (FMDV), a virus in the genus *Aphthovirus* within the family Picornaviridae. The disease is a very contagious disease in cloven-hoofed animals, and it affects animal species such as cattle, pigs, sheep, goats, elephants, mithuns, yaks, sambars, spotted deer, African buffaloes, antelopes, and wild boars.[2,3] The icosahedral virion of the FMDV comprises four capsid proteins: VP1, VP2, VP3, and VP4. The VP1 protein is a surface-exposed protein and contains the major antigenic determinants of the virus.[4–7] Five antigenic sites have been identified on the type O FMDV, and three of the sites (sites 1, 3, and 5) have been mapped at the VP1.[8] The amino acid residues that are critical for these antigenic sites are residues 144, 148, 154, and 208 for site 1; residues 43, 44, and 45 for site 3; and residue 149 for site 5.[9–11] Changes in these critical residues may lead to antigenic variation.

In 1997, a devastating FMD outbreak caused by the O/TW/97 strain occurred in Hsinchu County, Taiwan. The incursion resulted in severe economic losses. The FMDV strain shows a porcinophilic phenotype with a deleted nonstructural protein 3A gene.[12,13] Sequence analysis of the VP1 coding region showed that the viruses isolated in Taiwan between 1998 and 2009 were most similar to the O/TW/97 strain and to viruses isolated from Hong Kong and Vietnam in 1991—1996; this was also supported by phylogenetic analysis. Furthermore, substantial mutations were present in the viruses isolated in 2009. Some of these changes may result from vaccine pressure in the field. The serum neutralization test also supports that the viruses isolated in 2009 have significant changes in antigenicity.[12–14] The strain O/TW/99, which has a full-length 3A coding region, was isolated from subclinically infected cattle in 1999.[15]

When susceptible host animals are infected with FMDV, antibodies are elicited against viral structural proteins (SPs) and nonstructural proteins (NSPs). By contrast, animals administered inactivated FMD vaccines that lack or contain only trace amounts of NSPs are unlikely to induce NSP antibodies. Therefore, for FMD diagnosis, the NSP antibodies can be markers to differentiate infected from vaccinated animals (DIVA), providing the vaccine used is of high purity.[16] Detecting NSP antibodies has the additional advantage of being serotype-independent because the NSPs are predominantly conserved between the serotypes of FMDV.[17] Many methods of single signature assays for detecting NSP antibodies have been used, including agar gel immunodiffusion,[18] the latex bead agglutination test,[19] enzyme-linked immunoelectrotransfer blot,[20,21] enzyme-linked immunosorbent assay (ELISA),[4,18,22–28] and chromatographic strip assay.[29,30] For FMDV-SP diagnosis, different immunoassay formats have been established such as the virus neutralization test, liquid-phase blocking ELISA, solid-phase blocking ELISA, and competition ELISA.[4,31,46] Recombinant antigens and synthetic peptides derived from FMDV SPs and NSPs have been developed as an alternative to the inactivated virus antigen.[4]

Luminex assays are based on multianalyte profiling (xMAP) technology that simultaneously detects and quantifies multiple RNA or protein targets.[16,32–40] The xMAP system combines a flow cytometer, fluorescent-dyed microspheres (i.e., beads), lasers, and digital signal processing to multiplex up to 100 unique assays within a single sample efficiently. In the present study, a new application of xMAP was developed. To address the limitations of sensitivity and specificity to FMDV in existing antibody detection methods, xMAP was developed to detect and differentiate antibodies against the swine vesicular disease virus (SVDV). These tests were also compared with 3ABC polypeptide blocking ELISA and virus neutralization test (VNT) methods for the differential diagnosis and assessment of the immune status, respectively.

Materials and methods

Serum samples

To study the diagnostic sensitivity of the tests, sera were used from 32 eight-week-old specific pathogen-free (SPF) pigs experimentally infected with the FMDV-O/TW/97 strain. To compare the detection ability of the Luminex assay with that of other methods, the serum panel contained 320 swine sera that were collected sequentially from 32 eight-week-old SPF pigs (the ear tag numbers were 1513—1528, 1530—1533, 1536—1546, and 1548). The pigs were intradermally injected with 10^5 Tissue Culture Infectious Dose 50 (TCID50) (in a volume of 500 μL) of the FMDV-O/TW/97 strain into the heel bulb of the right foot. Blood samples were collected at 34 days postinfection (dpi). The sera in the panel were sampled at 0 days, 2 days, 4 days, 6 days, 8 days, 10 days, 14 days, 21 days, 28 days, and 34 days postinfection (dpi).

The performance of the xMAP in detecting SP and NSP antibodies against the FMDV-O/TW/99 strain was examined. Five 8-week-old SPF pigs (with ear tag numbers 1155-1, 1155-2, 1170, 1171, and 1172) were intradermally injected with10^5 TCID50 (in a volume of 500 μL) of the