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Diagnosis Based on 1D Gene Analysis of the Foot-And-Mouth Disease Virus

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ABSTRACT

A foot and mouth disease (FMD) is an acute, febrile and high contagious viral disease in many cloven hoofed domestic animals and more than 70 wild ones, resulting in severe finantial loss throughout the world. Choosing the correct seeds for foot-and-mouth disease (FMD) is an important issue in protecting this disease, and it is urgently necessary to establish a plan for vaccination in areas affected by FMD and to protect new foot-and-mouth disease. The genetic diversity and antigenic diversity of foot-and-mouth disease virus (FMDV) make eradication through vaccination difficult. Variable antigenic types exist in different geographic areas, and research projects on existing antigenic types are necessary to select vaccine in such an environment. From this, in this paper, oligonucleotide primers for detection and Selecting Serotype of FMDV were newly designed and synthesized. In addition, the nucleotide sequence of the 1D gene was aligned, the mutation region was determined, and the homology and phylogenetic relationship of the nucleotide sequence were analyzed. The antigenicity of the FMDV type O strains in the Democratic People's Republic of Korea and the correspondence between them were examined. The neutralization reaction was used to examine antigenicity between FMDV to select waxy seeds. To prevent FMD through this primer design and experimental method, the nucleic acid of FMDV was amplified by RT-PCR. Then, the nucleotide sequence of the 1D gene corresponding to the virus VP1 protein was analyzed and compared to select a seed vaccine strain.

Keywords : Foot and mouth disease (FMD), Vaccine, RT-PCR, ELISA

I. INTRODUCTION

This disease have severe economic consequences throughout the world due to herd destruction, trade restrictions, disease eradication costs, and market disruptions. FMDV is causing huge economic losses worldwide every year. The disease is characterized by fever, loss of appetite, ptyalism and vesicular

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eruptions on the feet, mouth and teats. A foot and mouth disease is an acute, febrile and high contagious viral disease in many cloven hoofed domestic animals and more than 70 wild ones[1]. The disease is caused by FMD virus (FMDV), which is a prototypic member of the genus aphthovirus. The office international des epizooties (OIE) reported that FMD ranks first among the notifiable infectious diseases of animals [2]. There are seven FMDV serotypes: A, O, C, SAT (South-African Territories) 1, SAT 2, SAT 3, and Asia1 based on the antigenicity of the capsid coating proteins [3]. Among them the most prevalent serotype in the world is "O" [4].The viral particle, or virion, contains a single-stranded RNA, which is roughly 8 500 nucleotides long. The most immunogenic protein is VP1, one of the structural proteins, which has got maximum exposure on the capsid surface. Genetic diversity and antigenic variability make FMDV difficult to control completely through vaccination. All the FMDV serotypes are clustered into genetic lineages distinctly with about 30–50% differences in the VP1 coding genes (capsid region genes) [5]. Outbreaks of FMD have been reported in large areas of Asia (including Middle East), Africa, and South America. During the last decade outbreaks of FMD have been occurred in Japan (e.g. Miyazaki Profecture), Hong Kong, Korean Peninsula, Mongolia, Russia and China. The outbreaks were caused by FMDV, which exhibited high homology to serotype O viruses isolated in Hong Kong, Korea and Russia in 2010 with nucleotide identities of 9 9.22, 98.9 and 98.59% respectively. This means that the FMD viruses circulating widely in the East Asia nations might be the same. Though the quarantine system was strictly applied in affected countries, improvement of global trade and rapid transport system often makes it impossible to prevent entry of the virus into the FMD susceptible population [6].Diagnosis and control of the disease has become difficult mainly due to presence of seven serotypes (and multiple subtypes) and strains. To success against FMD, continuous sero-monitoring, biannual vaccination and slaughter of seropositive animals is the key. Since 2010 when the FMD outbreak was observed in northeast Asia including Korean penninsula, China and Japan, these endemic countries have been vigorously monitoring the disease by sero-surveillance and genetic analysis, resulting in many useful reports. It is believed that the same strain caused outbreak throughout Asia. However, genetic diversity and antigenic variability of FMDV make it difficult to eradicate through vaccination. Presence of variable antigenic type in different geographical area and the coincidence of different antigenic type in a geographical area need the knowledge of existing antigenic type prior to start a control and eradication program or for the selection of a vaccine [7]. Within each serotype, there are many strains with antigenic diversity and hence enforce to incorporate more than one FMDV strain to attain a significant protection. All the FMDV serotypes are clustered into genetic lineages distinctly with about 30–50% differences in the VP1 coding gene (capsid region genes) [8]. New subtypes occasionally arise spontaneously. There is no cross protection between serotypes. Infection with one serotype type is fully susceptible with another six. Antigenic diversity led to variation in cross-protectivity particularly evident within the serotype A. Vaccines prepared from a single strain of serotype A virus may not provide immunity against other strains [9]. Further, variant forms (quasispecies) having versatility in antigenicity evolves in the field at different times due to high error rate during genome replication [10]. Like other RNA viruses, the FMDV has a high mutation rate as the RNA polymerase lacks the proof reading ctivity. The population size of FMDVs is large which is responsible for high antigenic variability together with continuous circulation of the field virus and plasticity of the major neutralizing sites on surface of the virion. They give rise to serious problems inspite of availability of good inactivated vaccine [11]. Therefore, in this paper, based on the detailed analysis of the precedence literature, we specifically studied methods for detecting FMDV and selecting serotypes, aligning the nucleotide sequence of 1D gene, and determining the mutant region.

II. METHODS AND MATERIAL

2.1 Materials

Virus: FMD virus seed vaccine (O, A)
Strain separated from the pathologic material in the proper areas.
Reagent: trisol, chloroform, isopropyl alcohol, ethanol, dNTP mixture, PCR primer, Reverse transcriptase, TaqDNA polymerase, MgCl2, Agarose gel, ethidium bromide, buffer solution
Instrument and equipment: PCR reactor, centrifugal separator, Bordex, Electrophoresis, UV spectrometer, micro pipet
Experimental animal: cattle, pig

2.2 Methods

- Base sequence analysis

The oligonucleotide primers for the detection and serum differentiation was designed and composed by the opened data of the NCBI and publication. Extraction of template RNA was done by a Trisol-LS method.

RT-PCR is progressed at two stages.
PCR program for the cDNA synthesis and nucleic acid amplification was as following;
It was synthesised for 30 minutes at 42 ℃ and denaturated for 3 minutes at 94℃.

- Vaccine matching test by the gene analysis.

DNA Star program was used for DNA analysis.
The base sequence of 1D gene was arranged and the variation areas were determined. So we analysed the homology of base sequence and pedigree relationship and estimated the price of the seed vaccine.

- Vaccine matching test by the serum analysis.

It was conducted to serum neutralization test and a LPB-ELISA method.(Liquid Phase Blocking ELISA (LPBE))

The relation of antigenic similarity was done by the methods of investigating the correlation coefficient according to guide book of the international veterinary secretariat, it was compared with gene analysis and estimated the value of seed vaccine.

If the correlation coefficient is ranged from 0.4 to 1.0, the field separation strain and the vaccin strain was closely related to the antigen and the seed vaccine strain could provide protective immunity for infection that would protect the field strain.

If the correlation coefficient is ranged from 0.2 to 0.39, these were related to the antigen to some extent. Also it couldn be vaccine strain without more relative seed vaccine,

If it was ranged less than 0.2, the field separation strain has comparatively long relation to a vaccine strain and the seed vaccine couldn't provide the protective immunity for infection that could protect the field strain.

III. RESULTS

3.1 Base sequence analysis by the RT-PCR products.

To check antigenicity and their matching test between the types O of seed foot and mouthdisease virus in our country, first of all we completely analyzed the base sequence of 1D gene related to antigenic similarity among 3 kinds of FMD virus strains and amino acid arrangement ofVP1 protein forecasted from the base sequences.

As a reference, we analyzed Manisa strain that had a wide antigenic width.

Table1. Completely analyzed base sequence of the live-attenuated virus 1D gene

```
ACCACCTCCCCGGGTGAGTCAGCTGACCCTGTGACCGCCACCGTTGAGAACTACGGTGGTGAGACACAGGTCCAGAGACGCCAACACACA     90
GACGTCTCGTTCATTTTGGATAGATTTGTGAAAGTGACGCCAAAAGATCAAATTAATGTGTTGGACCTGATGCAGACCCCTGCACACACG    180
CTCGTGGGAGCGCTCCTGCGTACTGCCACTTACTATTTCGCTGACTTAGAAGTGGCAGTGAAGCACGAGGGAAACCTCACATGGGTCCCA    270
AACGGGGCGCCTGAAGCGGCACTGGACAACACCACCAACCCAACAGCATACCACAAGGCACCACTTACCCGGCTTGCTTTGCCGTACACG    360
GCACCACACCGCGTGTTGGCAACTGTTTACAACGGAAACTGCAAGTATGGTAGTGGTCCAGTGCCCAATACAAGAGGTGACCTCCAAGTG    450
TTGGCCCAGAAGGCAGCGAGACCGCTGCCCACCTCCTTCAACTATGGTGCTATCAAAGCCACTCGGGTGACTGAACTGCTTTACCGCATG    540
AAGAGGGCTGAAACATACTGCCCCCGACCTCTTTTGGCCATACATCCGAGTGAGGCTAGACACAAACAAAAGATTGTGGCACCTGTGAAA    630
CAGCTCCTG                                                                                      639
```

Table2. Completely analyzed base sequence of the Manisa strain's (AY593823VP1)1D gene

```
actacctccgcgggcgagtcagctgaccccgtgaccgccaccgttgagaattacggtggcgagacacaggtccagaggcgccaacacacg     90
gacgtctcatttatattagacagatttgtgaaagtgacaccaaaagaccaaattaatgtattggacctgatgcaaacccctgctcacact    180
ttggtgggagcactccttcgtactgccacttactatttcgctgacttagaggtggcagtgaagcacgagggaaacctcacctgggtcccg    270
aacggggcgcctgaagcggcgttggacaacaccaccaacccaacagcttaccacaaggcaccactcacccgacttgcactgccttacacg    360
gcgccacaccgcgtgttggctactgtttacaacgggaacagcaagtatggtgacggcacggtggccaatgtgagaggtgacctgcaagtg    450
ttggcccagaaggcggcgagagcgctgcctacctccttcaactacggtgccattaaagctactcgggtgactgaactgctttaccgcatg    540
aagagggctgagacatactgtccccggcctcttttggccattcacccggaccaggctagacacaagcagaagattgtggcaccggtgaaa    630
cagcttct                                                                                       638
```

Table3. Completely analyzed base sequence of Manisa strain's (FDI251477-1VP1)1D gene

```
actacctccgcgggcgagtcagctgaccccgtgaccgccaccgttgagaattacggtggcgagacacaggtccagaggcgccaacacacg     90
gacgtctcatttatattagacagatttgtgaaagtgacaccaaaagaccaaattaatgtattggacctgatgcaaacccctgctcacact    180
ttggtgggagcactccttcgtactgccacttactatttcgctgacttagaggtggcagtgaagcacgagggaaacctcacctgggtcccg    270
aacggggcgcctgaagcggcgttggacaacaccaccaacccaacagcttaccacaaggcaccactcacccgacttgcactgccttacacg    360
gcgccacaccgcgtgttggctactgtttacaacgggaactgcaagtatggtgacggcacggtggccaatgtgagaggtgacctgcaagtg    450
ttggcccagaaggcggcgagagcgctgcctacctccttcaattacggtgccattaaagctactcgggtgactgaactgctttaccgcatg    540
aagagggctgagacatactgccccggcctcttttggccattcacccggaccaggctagacacaagcagaagattgtggcaccggtgaaa    630
cagcttct                                                                                       638
```

Table4. Completely analyzed base sequence of the type pan-Asia O strain's (2006)1D gene

```
accacctccacaggtgagtcggctgaccccgtgactgccactgttgagaactacggtggtgagacacaggtccagagacgccaacacacg     90
gatgtctcgttcatattagacagatttgtgaaagtaacaccaaaagaccaaattaatgtgttggacctgatgcaaacccctgcacacact    180
ttggtaggcgcgctcctccgtactgccacctactacttcgcagatctagaagtggcagtgaaacacgaggggaaccttacctgggtcccg    270
aacggggcgcccgagacagcgttggacaacaccaccaatccaacggcctaccacaaggcaccgctcacccggcttgcactgccttacacg    360
gcaccacaccgtgtcttggctactgtttacaacgggaactgcaagtatggcgagggccccgtgaccaatgtgagaggtgacctgcaagta    450
ttggcccagaaggcggcaagaacgctgcctacctccttcaactacggtgccatcaaagccactcgggtgactgaactgctttaccgcatg    540
aagagggccgaaacatactgtccccggcctcttttggctattcacccgagcgaagctagacacaaacaaaagattgtggcgcctgtgaaa    630
cagcttctg                                                                                      639
```

Table5. Completely analyzed base sequence of type pan-Asia O strain's (AY114146)1D gene

```
accacctccacaggtgagtcggctgaccccgtgactgccaccgttgagaactacggtggtgagacacaggtccagagacgccaacacacg     90
gatgtctcgttcatactagacagatttgtgaaagtaacaccaaaagaccaaattaatgtgttggacctgatgcaaatccctgcacacact    180
ttggtaggcgcgctcctccgtactgccacctactacttcgcagatctggaagtggcagtgaaacacgaggggaacctcacctgggtcccg    270
aacggggcgcccgaggcagcgttggacaacaccaccaatccaacggcctatcacaaggcgccgctcacccggcttgcactgccttacacg    360
gcaccacaccgtgtcttggctactgtttacaacgggaactgcaagtatggcgagagccccgtgaccaatctgagaggtgacctgcaagtg    450
ttgacccagaaggcggcaagaacgctgcctacctccttcaattacggtgccatcaaagccactcgggtgactgaactgctttaccgcatg    540
aagagggccgaaacatactgccccggcctcttttggctattcacccgagcgaagctagacacaaacaaaagattgtggcgcctgtgaaa    630
cagctgttg                                                                                    639
```

Table6. Completely analyzed base sequence of southeast asia field strain (2011)1D gene

```
accacttcgacaggcgagtcggctgaccccgtgactgccaccgttgagaattacggcggcgagacacaggtccagaggcgccaccacaca     90
gacgtctcattcatattggacagatttgtgaaagtcacaccaaaagactcaataaatgtattggacctgatgcagacccctcccacacc    180
ctagtaggggcgctcctccgcactgccacttactatttcgctgatctagaggtggcagtgaaacacgaggggacctcacctgggtgcca    270
aatggagcacctgaagcagccttggacaacaccaccaacccaacggcgtaccataaggcgccgcttacccggcttgcattgccctacacg    360
gcaccacaccgtgttttggccaccgtttacaacgggaactgcaaatacaccgggggctcactgcccaatgtgagaggcgatctccaagtg    450
ctggctcagaaggcagcgaggccgctgcctacttctttcaactacggtgccatcaaagccactcgggtgacagaactgctgtaccgcatg    540
aagagggccgagacgtactgtcctcggcccctcttggctgttcacccgagtgcggccagacacaaacagaaaatagtggcacctgtaaag    630
cagtccttg                                                                                    639
```

Table7. Completely analyzed base sequence of the normal MAY-98(FDI303521) strain 1D gene

```
accacttcganaggcgagtcggctgaccccgtgactgccnccgttgagaactacggtggtgagacacaggnccagaggcgccaccacaca     90
gacgtctcattcatattggacagatttgtgaaagttacaccaaaagaccaaattaatgtgcnggacctgatgcagacccccccccacacc    180
ctggtgggggcgctccttcgtactgccacttactatttcgctgatctagaagtggcagtgaaacacgaggggggacctcacctgggtgccg    270
aatggancacctgaggcagctttgaacaacaccaccaacccaacggcgtaccacaaagcgcngctcacccggcttgcactgccctacacg    360
gcaccacaccgtgttttggctaccgtttacancgggaactgcnaatacgccgagggctcactgaccaacgtgagaggtgatctccaggtg    450
ctggctcagaaggcggcgaggccgctgcctacttctttcaactacggtgccatcaaagccactcgggtgacagaactgctgtaccgcatg    540
aagagggccganacatactgtcctcggcctctcttggctgtccatccggatgaggctagacacaaacagaaaatagtggcacctgtgaag    630
cagtccttg                                                                                    639
```

As we can see from Table 1 to 7, completely analyzed base pair of the different strain's 1D gene was about 638 to 639.

3.2 Base sequence homology and pedigree relationship between 1D genes of the FMD virus type O strain

To determine the variability of base sequence of 1D gene, the main gene that determines the antigenic similarity, its homology and pedigree relationship was investigated.

Table 8. Base sequence homology of 1D genes of the FMDV type O strains

	1	2	3	4	5	6	7	
1		89.2	89.3	87.9	87.3	83.4	83.1	1
2	11.9		99.5	90.1	88.9	83.7	84.3	2
3	11.7	0.5		90.0	89.3	83.5	84.2	3
4	13.4	10.8	11.0		97.5	85.3	86.2	4
5	14.2	12.4	11.8	2.6		84.5	85.8	5
6	19.3	19.0	19.2	16.8	17.8		92.2	6
7	18.1	16.5	16.8	14.0	14.6	6.9		7
	1	2	3	4	5	6	7	

1 vaccine strain(VP1).seq
2 manisa AY593823VP1.seq
3 manisa FDI251477-1VP1.seq
4 field virus(2006VP1).seq
5 panAsia-O VP-1 (AY114146 partial).seq
6 field virus(2011).seq
7 MYA-98-VP1 (FDI303521 partial).seq

As shown in the Table 8, living virus had the highest homology with Manisa strain (89.2 ~ 89.3 %), middle level in homology (87.3~87.9%) with pan-Asia type O and lowest homology (83.1~83.4%) with Mya-98 strain. Thus living virus and pan-Asia type O had comparatively high relationship with Manisa strain.

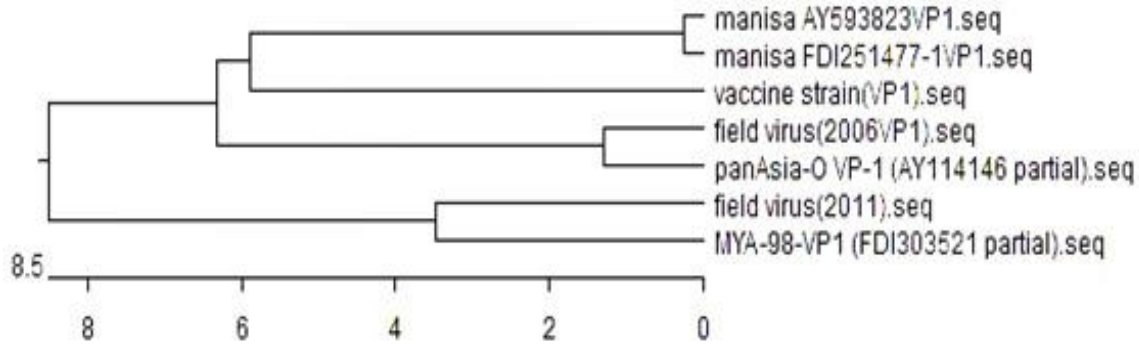

Figure 1. Pedigree relationship between base sequences of 1D gene

As shown in Fig 1, living virus had a tiller of the system like Manisa strain, panAsia type O and field strain in 2006 had a tiller of equal pedigree, and Mya-98 and field strain in 2011 were equal.

3.3 Vaccine matching test by serotype analysis.

To select the seed vaccin in our country, match test of antigenic similarity between FMD viruses was progressed in the neutralization reaction. The results are as following.

Table9. Matching test of FMD viruses by neutralization reaction

Animal kind	type of antigen	serotype	Mean serum Neutralizing antibody titer (2^x)	correlation coefficient (R)
cattle	FMDV strain (2010)	FMDV strain (2010)	10.48±0.43	1.00
		Mya-98	6.84±0.41	0.65
		Pan-asia O	9.33±0.57	0.89
		Manisa	7.64±0.32	0.72
swine	FMDV strain (2011)	FMDV strain (2011)	10.23±0.36	1.00
		Mya-98	8.64±0.22	0.84
		Pan-asia O	7.18±0.35	0.70
		Manisa	5.60±0.18	0.54
swine	FMDV strain (2014)	FMDV strain (2014)	10.15±0.27	1.00
		Mya-98	8.81±0.25	0.86
		Pan-asia O	7.34±0.19	0.72
		Manisa	5.73±0.15	0.56

As we can see in the table 9, because the correlation coefficient is in 0.54~0.89, FMD virus type O strains was related to antigen and these can give crossing immunity to some extent.

Next, we conducted matching test of antigenic similarity between different FMD viruses by LPB-ELISA (Table 10).

The results are below.

Table10. Matching test between FMD viruses by LPB-ELISA ways

Antigen type	Animal type	Serotype	Mean serum Neutralizing antibody titer (2^x)	correlation coefficient (R)
Mya-98	cattle	Mya-98	9.4±0.8	1.00
		Pan-asia O	7.6±0.5	0.80
		Manisa	5.4±0.5	0.57
	swine	Mya-98	8.4±0.8	1.00
		Pan-asia O	6.6±0.8	0.78
		Manisa	5.0±0.7	0.59

As you can see in table 10, because correlation coefficient was in 0.57-0.8, FMD virus type O strains is related to antigen and these can give a crossing immunity to some extent.

IV.Discussion

In this paper, the genetic diversity and antigenic diversity of FMDV were used to analyze antigenic types present in different geographic regions, and based on this, the selection of seed vaccine strain was analyzed. The analysis confirmed that it was possible to select a seed vaccine strain that could prevent the spread of FMD and eradicate it within a short time.If the relative side number is between 0.4 and 1.0, the Wild virus strain and the seed vaccine strain are closely related antigenically, and the seed vaccine strain can provide infectious defense immunity to block the Wild virus strain. In addition, if the number of relative side counts is 0.2~0.39, the Wild virus strain and the seed vaccine strain are antigenically related, and if there is no more closely related seed vaccine strain, it can be used as a seed vaccine strain. If the number of relatives is less than 0.2, the outdoor tree has a relatively distant relationship with the seed vaccine strain, and the seed vaccine strain does not provide the infection defense immunity that can block the Wild virus strain. In the thesis, the nucleotide sequence of the 1D gene was aligned, the mutation region was determined, and the homology of the nucleotide sequence and the phylogenetic relationship were analyzed. In addition, the FMDV type O strains have a certain level of cross immunity to each other because they have a certain relationship to each other. By confirming that it can be given, it gave a theoretical and practical basis for selecting seed vaccine strain with high reliability.

V. CONCLUSION

Foot-and-mouth disease virus (FMDV) has sensitivity and specificity, so detection of FMDV generally proceeds in parallel with RT-PCR, ELISA, and virus isolation. In this paper, the method of selecting a seed vaccine strain that can eradicate FMDV using RT-PCR was specifically mentioned. The method of gene base sequence analysis of FMDV proposed in this paper has confirmed through experiments that it is possible to select a seed vaccine strain that can prevent the spread of FMD and eradicate it within a short time. The nucleotide arrangement of the 1D gene corresponding to the virus VP1 protein was analyzed and compared to select a seed vaccine strain. In order to select theseed vaccine strain, an antigenic conformance review test between FMDV was conducted as a neutralization reaction, confirming that FMD virus type O strains had a certain antigenic relationship and could give a certain level of cross immunity to each other.

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