



**Study on digestive and respiratory viral pathogens of Korean  
native black goat:**

- 1. Establishment of serum bank for epidemiological study**
- 2. Isolation of viral pathogens from digestive and respiratory  
tracts**
- 3. Development of vaccine to the isolated virus**

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II.

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. IMF

2

가 가

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67

가 가

78.8%

가

*Salmonella spp.*

가

rotavirus coronavirus, border disease virus picornavirus

pestivirus lentivirus Maedi-Visna virus

goat small pox virus

가

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가

가

가

### III.

1(1997- 1998),  
2(1998- 1999)                      3(1999- 2000)  
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1) 1 :                      .

78.7%

가  
*Salmonella spp.*가

가

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가

genotyping VP4, VP7 NSP4

, RNA-RNA hybridization

genotyping

3)

3

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가

formalin BEI

1 1 TF104  
100

#### IV.

#### 가.

#### 1.

가) 200

44%, 26%, 8%

)

)

respiratory syncytial virus: BRSV)

diarrhea virus: BVDV)

BRSV , BVDV

)

2.

가)

)

가

TF104

)

7 37 , 6 18 , 2 2 , 5 23 ,  
5 25 , 2 7

가 가 0 512 79%

100%

)

가

2

가

2

106T CID50M0

가

1 5

가

,

2 10

1 8

1

7

7

가

가

2

1 4

6

2

)

TF104

77

5



5 3 1 가 .

5 3 77

) VP4 C486,  
RRV 2,328 776

) VP4 VP4  
type NCDV, UK, b223, 993/83, OSU, MDR13,  
L338, Eb, Lp14 rotavirus VP4 sequence homology  
type 1 NCDV 71/75%, type2 SA11  
79/88%, type 3 RRV가 83/91% , type 4  
L26 72/71%, type 5 UK 72/76%, type 6 1076  
72/72%, type 7 OSU 75/80%, type 8 Wa  
72/71%, type 9 K8 70/69%, type 10 69M 76/85%,  
type 11 B223 60/81%, type 12 B2 75/81%, type 13  
MDR13 75/78%, type 14 Mc35 70/70%, type 15  
Lp14 76/82%, type 16 Eb 71/76%, type 17  
993/83 67/63%, type 18 HALL1166 70/71%, type 19  
L338 75/78% . type 3 RRV  
83/91% type 3 가

) VP7 978  
326

) VP7 type  
NCDV, KK3, OSU, YM, RRV, SA11,  
Wa, Gottfried, HCR3, L338, F123, Eb,  
Ch2 sequence homology . type 1

Wa 75/81%, type 2 S2 75/75%, type 3 Eb  
 77/89%, HCR3 85/95%, RRV 76/81%, SA11 83/95%, type 4  
 Gottfried 75/78%, type 5 OSU 78/85%, type 6  
 NCDV 77/85%, type 7 Ch2 67/59%, type 8  
 B37 75/80%, type 9 116E 77/85%, type 10 KK3  
 77/83%, type 11 YM 78/88%, type 12 L26  
 75/81%, type 13 L338 77/82%, type 14 F123  
 83/87% . type 3 Eb, HCR3, RRV, SA11  
 76- 85/81- 95% 가 type  
 3 가 .  
 ) NSP4 525  
 175 .  
 ) NSP4 type  
 NCDV, UK, YM, RRV, SA11, Wa,  
 AU- 1, E210, E201, S2, FRV1  
 phylogram , FRV1, AU1, RRV가  
 type .  
 ) 가 NSP4 type 3 ,  
 conserved NSP4 sequence VP4  
 binding domain 131- 148 RRV, FRV1, AU- 1, M37,  
 Wa, NCDV, SA11, UK  
 NSP4 type AU- 1, FRV1  
 RRV 18 M37 Wa 13 가 ,  
 NCDV UK 10가 SA11 6  
 RRV, RRV1, AU- 1  
 group .  
 ) RNA- RNA hybridization genotyping GRV probe  
 Wa, Ds- 1, 69M, R2, P, SA11, RRV, FRV- 1,

NCDV, OSU hybridization Wa, Ds-1, 69M, SA11,  
 NCDV, OSU hybrid가 , RRV 6-7 , AU-1  
 6 , FRV-1, R2, RS15 5 , R2, FRV64 4 hybrid가 .  
 RRV probe RRV, FRV64 hybridization 5 6 hybrid  
 , FRV64 GRV hybridization 5 hybrid가  
 . GRV probe  
 AU-1, FRV-1, FRV64, RRV가 group GRV가 .

### 3.

가) 106 TCID<sub>50</sub> 19  
 , 12 가  
 .  
 , 가  
 ) , M<sub>0</sub> 104 TCID<sub>50</sub>가 100  
 95  
 5  
 ) , 19 8  
 가 , 2  
 6 2 8  
 ) 7 가



, 가

20%

( )

가

,

가

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가

가

가

## Summary

It has been suggested that most economical losses of black goat industry are caused by digestive and respiratory diseases. Therefore, we have performed a project regarding to goat diseases and its epidemiological studies.

The first study of this project included the disease status of Korea and the serological survey of the viral diseases. The second study was the isolation of the virus from the digestive and the respiratory tracts. Finally, we developed the live vaccine against the isolate.

First of all, we surveyed most of the farms which house over 200 goats and we took the disease histories for the report. Then, we took blood to carry out serological surveys of the disease. We found out that there were 44% of respiratory disease, 26% of digestive disease and 8% of ocular disease. According to this survey, most of the farms seemed to be infected by a sort of bovine respiratory syncytial virus, but not by the same family of bovine diarrhea virus and foot & mouth disease (FMD).

To investigate the major pathogens of digestive diseases of domestic black goats, we tried to identify the causative agents in fecal specimens from diseased black goats. We could isolate the virus, and it was identified as rotavirus since the monoclonal antibody to VP8 protein of bovine rotavirus was reacted with the isolate, cultured on TF104 monolayers. However, pathogenic bacteria were not isolated, indicating that the digestive diseases in black goats are predominantly caused by virus. Serological survey of goat rotavirus indicated that most of the black goats were infected with rotavirus.

To characterize the black goat rotavirus (GRV), we sequenced genes of VP4, VP7 and NSP4 and those nucleotide sequences were compared with those of already known rotaviruses. The result revealed that the genotype of the isolate was closely related with G3P[3]. The phylogram analysis of NSP4 GRV showed that the genogroup of GRV belongs to the group of human (AU-1), feline (FRV-1) and monkey rotaviruses (RRV) rather than to that of bovine rotavirus. To see where GRV was originated, RNA-RNA hybridization was performed. GRV possessed six gene segments that formed hybrids with human rotavirus (AU-1), monkey rotavirus (RRV) and five gene segments that formed hybrids

with feline rotavirus (FRV-1) and canine rotavirus (RS-15). The data strongly indicated that GRV infection to goats may come from cats or dogs. These results supported the view that rotavirus may cross the species barrier whereby animal rotaviruses can infect human and vice versa.

To develop the rotavirus vaccine, the isolate was blind-passaged on TF104 cells 100 times. The attenuated virus of  $10^6$  TCID<sub>50</sub>/ml was inoculated into 19 goats. Twelve of them showed diarrhea or abnormal feces. To reduce the pathogenicity of the virus, the viral titer of the inoculated vaccine was reduced to  $10^4$  TCID<sub>50</sub>/ml and the vaccine was added in the drinking water. One hundred of goats were orally vaccinated and their fecal status was monitored weekly. Ninety five goats showed no sign of illness and the rest of them had diarrhea. Seven of the ninety five healthy goats were challenged with virulent rotavirus. All of them had no sign of diarrhea.

In conclusion, we can confirm that this live-attenuated vaccine is useful to prevent goats from rotavirus infection in the field.

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1        가

1.	-----	1
2.	-----	2
3.	-----	3

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1

1.	-----	10
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3.	-----	13

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2.	-----	18

3.			
1)		가	
	가	-----	20
2)		-----	21
III.			
1.		-----	23
2.		-----	24
3.			
1)		-----	26
2)		-----	26
4			
1.		-----	28
2.		-----	32
3.			
1)	VP4	-----	38
2)	VP4 p type	-----	45
3)	VP7	-----	48
4)	VP7 type	-----	52
5)	NSP4	-----	54
6)	NSP4 phylogram	-----	58
7)	NSP4 VP4 binding domain sequence	----	59
8)	RNA-RNA hybridization genotyping	-----	60
3			
1.		-----	64
2.		-----	68

3.

- 1) -----70
- 2) -----70
- 3) -----72
- 4) -----72
- 5) -----73

4 -----75

5 -----85

# 1 가

가

가

1994; 1984). ( , 1996; , 1994; , 1994; 1984).

78.8%

가

*Salmonella spp.*

( , 1995).

가

rotavirus corona virus, border disease virus picornavirus

pestivirus lentivirus Maedi-Visna virus

goat small pox virus (Timoney ,

1988).

가

1.

, BVDV FMDV 가  
가 96 2  
100 TCID<sub>50</sub> well 37 1  
10% Fetal bovine serum -MEM EbTr  
MDBK well 105 37 3 5  
가 well  
가 .

2.

가 가 ELISA  
. ELISA , plate  
well  
1 , PBS , horse reddish peroxidase(HRP)  
anti- goat antiserum 1:1000 PBS plate well  
. 1 ELISA reader OD

3.

Nasal swab incubator 24  
, , API card .

44%, 26%, 8%

1-1

가

1-4, 1-5 Bovine

viral diarrhea virus Bovine respiratory syncytial virus

1-2

가

가

(

1-1). 가 가

Shewanella putrefaciens,  
Burkholderia cepatica, Kinella denitrifican, CDC-group II, Acinetobacter  
radioresistens, Kingella kingae .

2000 가

1997

ELISA 2 가

( 1-6).



1-1.

		5					
		3	6	2		1	
		2	6				
		3	3				
		1	9				3
		4	1				
		2	1				
		2	1			5	
		2					
		1	3			1	2
							1
		1	3	1			2
		3	8	1			4
		1	2	3			
			11	1			
		4					1
		1	1	1			
		3		2	1	2	3
			8	1		2	3
(%)		37(26)	63(44)	12(8)	1(1)	11(8)	19(13)



1-1. : ,  
가 , 가 .  
가 .



1-2.

:



1-3. : 가  
, .  
.

1-2. 가

		가 ( )
		6
		4
		10
		6
		7
		3
		6
		-
		-
		8
		-
		2
		4
		4
		3
		9
		10
		8
		5
		95

1-4. Serological survey of bovine respiratory syncytial virus infection



2

1

VP4 dsRNA

가 가

- strand , dsRNA

subviral particles , free dsRNA free - strand ssRNA

particle , subviral particle viroplasm

가 가

(Kapikian

1996), 가 (Estes

1989), 가

sialic- acid 가

(Ciarlet 1999, Fukudome 1989, Keljo 1988, Mendez

1993), sialic acid

(Mendez 1993). Rhesus 가

sialic acid 가 VP5

(Zarate 2000).

2 1 integrin ,

VP4 DGE sequence가  
 (Coulson 1997, Marilyn 2000).  
 , viral transcriptase  
 (Shahrabadi Lee 1986, Shahrabadi 1987). replication cycle  
 가 가 (Hewish 2000),  
 가 .  
 가 .

1.

200  
 ,  
 - 70 .

2.

NCDV, C486, OSU, RRV,  
 SA11, AU-1, Wa, DS-1, H123, 69M, P, R2, RS15, FRV-1, FRV64  
 .

3.

TF104 10% Fetal calf  
 serum (Biofluid) gentamycin (50  $\mu\text{g}/\text{Ml}$  Amnesco) - MEM (Gibco,  
 BRL) .



4.

200 가  
 가  
 가 PBS 1:5 0.2  $\mu\text{m}$   
 가 10  $\mu\text{g}/\text{Ml}$  trypsin  
 1 5% CO<sub>2</sub>, 37 PBS 3  
 0.5  $\mu\text{g}/\text{Ml}$  trypsin 가 -MEM (CPE, cytopathic  
 effect)가  
 48 72 3  
 4000  $\times$  g 15 -70

5.

-20  
 Microtome slide glass acetone  
 5  
 6 well plate coverslip  
 TF104 cell 1 1, 2  
 CPE가 CPE가 PBS 3  
 5 acetone 1  
 VP8  
 PBS 3 10 FITC conjugated rabbit anti-mouse IgG  
 1 PBS 3 10

6.

TF104 3

, 2500 rpm 25  
 25ml 30% sucrose cushion pasteur pipette  
 5ml 4 25,000rpm 2 30  
 500 $\mu$  complete  
 Freund's adjuvant Balb/c 10 가  
 7  
 myeloma cell line SP/0 , 96 well plate  
 , CO2 incubator 1  
 hybridoma가  
 well hybridoma . 105 hybridoma cell  
 incomplete Freund's adjuvant 10  
 가 가  
 ,  
 가

VP8 가  
 (Fig. 2-1-1). 37 1  
 TF104 16 CPE가  
 , VP8  
 (Fig. 2-1-2).  
 가 ( 2-1-1).

Fig.2-1-2

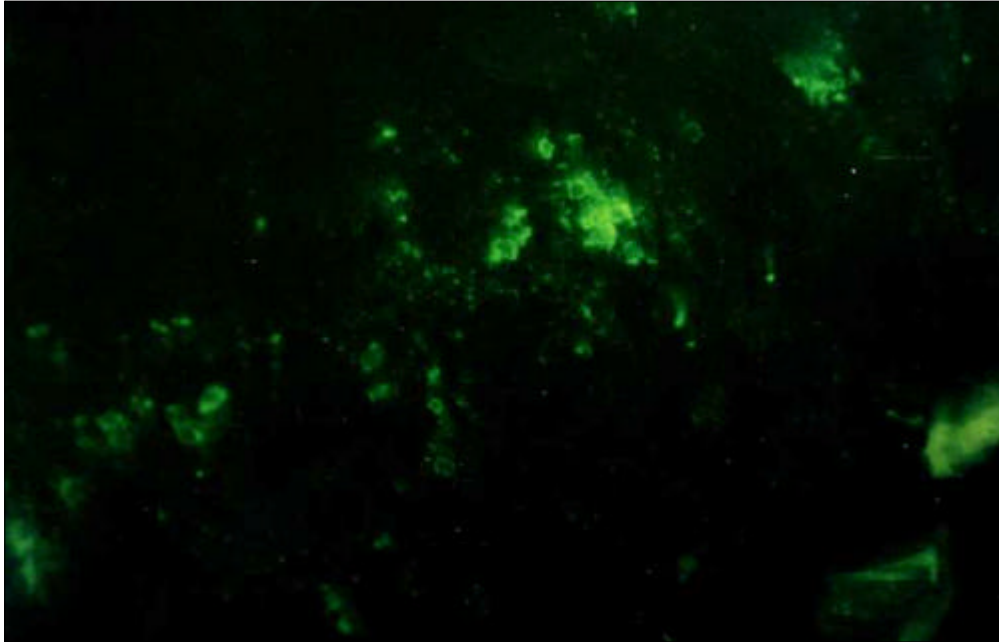


Fig. 2-1-1. Detection of goat rotavirus using monoclonal antibody against bovine rotavirus. Small intestine of diarrheal goat was frozen at -20 . Tissue was sectioned using microtome and applied with monoclonal antibody against bovine rotavirus VP8. The tissue slide was washed several times with PBS. The tissue slide was applied with rabbit-anti-mouse IgG conjugated with FITC and observed using fluorescent microscope.

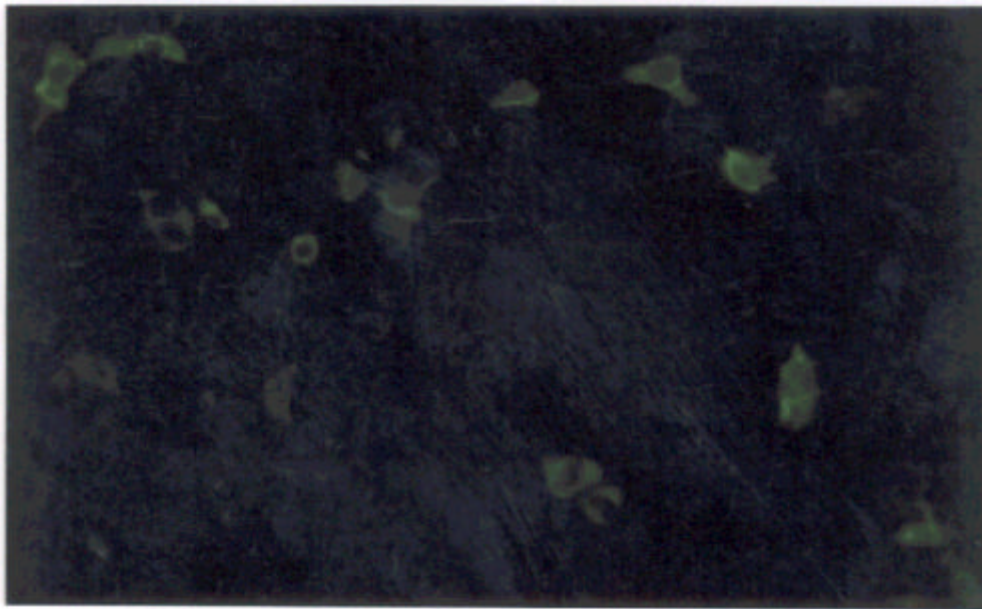


Fig. 2-1-2. Confirmation of goat rotavirus using monoclonal antibody to bovine rotavirus. Intestinal tissue was ground in PBS using sea sand. The ground sample was centrifuged and its supernatant was applied on the monolayered TF104 cells on the cover slip. The cover slip was washed three times with PBS and then fixed with absolute acetone. The slide was stained with monoclonal antibody in parallel with rabbit anti-mouse IgG conjugated with FITC. The slide was observed under the fluorescent microscope.

2-1-1.

Monoclonal antibody	Activity of hyridoma supernatant	
	FA	HI(titer)
A21-5	+	8
D6-3	+	16
A10-8	+	0
A14-1	+	0
A7-5	+	0
B15-4	+	0
B17-1	+	0
B61-3	+	0
D27-12	+	0

(Estes 1996),  
 가 , 20 50%가  
 . 4  
 (Kapikian 1989). 1  
 5% 7  
 (Ratafia 1987). group A  
 1 2 8 16 36% (Gomwalk  
 1988) 6 가 (McNulty Logan, 1983),  
 3 4 가 2 6  
 (Gelberg 1991, Utrera 1984). 3  
 , 4 (Tizipori 1981).  
 48.6%, 28.6% (Kaminfolo 1994).  
 가  
 (Mendes 1994, Munoz 1996, Mendes 1994).  
 , 90  
 (Blacklow Greenberg 1991) 가  
 , 100 가  
 , 50%가  
 5  
 (Kapikian Chanock 1996, Gurwithet 1981, Mata 1983).  
 (Hurst 1980),  
 가 ,

10 °  
(Cook 1990).

가

1.

가 96 2 10µg/Ml  
trypsin 37 30 100TCID50 well  
37 1 , 10% Fetal calf serum  
- MEM TF104 가 plate  
2 3 CO2 incubator CPE가  
가 CPE가

2.

2 0.2% bovine serum albumin-PBS  
O 가 vortexing 3  
가

3.

10% Kaolin- PBS  
2 . PBS 8HA unit가  
microplate well 가 vortexing  
1 0.2% Bovine serum albumin-PBS RBC

가 0.25% (v/v)가

microplate

well

가

3

가

.



1.

가

가

2-2-1

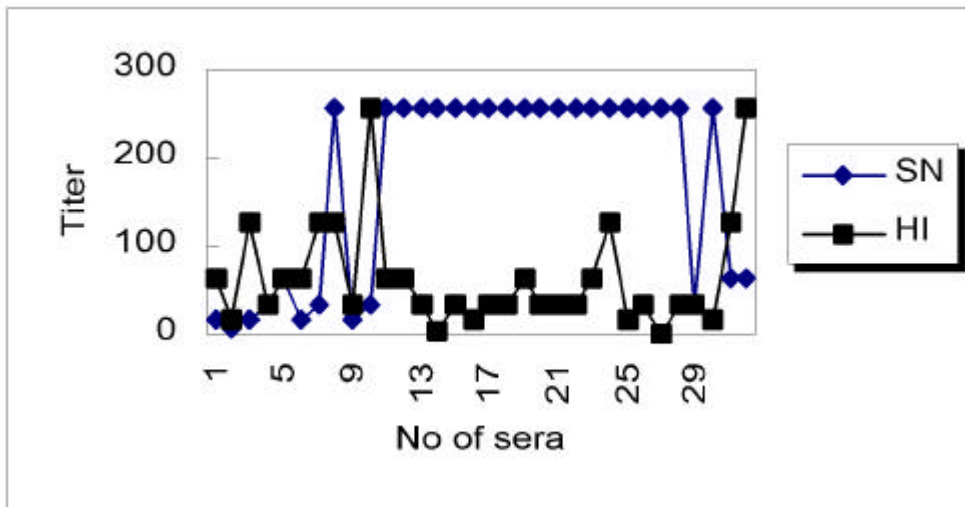
가

가

가

가

가



2-2-1.

가

가

2.

가 . 2-2-1 7  
37 0 256 가 94% ,  
6 18 32 512 가 100% ,  
2 2 16 128 가 100% .  
5 23 0 256 가 85% , 5  
25 0 256 가 90% , 2 7  
16 256 가 100% , 4 17 0  
512 79%

2-2-1.

		가 ( )	(%)
		8 128(4)	100
		16 64(6)	100
	1	0 256(5)	80
	2	32 256(5)	100
	3	0 512(5)	80
		32 512(6)	100
		32 256(6)	100
		0 512(37)	94
	1	64 256(2)	100
	2	32 256(4)	100
		512(3)	100
		32 256(6)	100
		512(1)	100
		32 64(2)	100
		32 512(18)	100
		128(1)	100
		16(1)	100
		16 128(2)	100
		32 256(5)	100
	1	128(4)	100
	2	32 256(5)	100
	1	0 32(4)	75
	2	0 64(4)	50
		0 256(23)	85
		4 32(2)	100
		0 32(6)	50
	1	16 32(2)	100
	2	2 256(7)	100
		32 128(4)	100
		0 256(25)	90
		16 64(2)	100
		16 256(5)	100
		16 256(7)	100
	1	0 512(7)	85
	2	64 512(4)	100
		512(3)	100
		0 512(3)	33
		0 512(17)	79
		0 512(129)	93

(Holmes 1975, Moon 1994).

(Starkey 1986).

3가

가

가

(Davidson 1977, Graham

1984). Na+K+ ATP glucose-coupled Na+

Na+

(Davidson 1977, Graham 1984).

, NSP4

NSP4

(Au 1989). , NSP4

(Au 1989).

aa 114- 135 peptide

가

(Ball 1996). aa 114- 135

peptide

NSP4

domain

system (ENS) 가 (Cassuto 1981), ENS

(Lundgren 2000).

ENS (Brunsson 1994,) 가 Primary neuron (Weclawicz 1998), toxin NSP4 가 (Peregrin 1997).

1.

3 4 10<sup>6</sup>TCID<sub>50</sub>/Ml 10Ml

2.

1ml 가 10<sup>6</sup>μg/Ml trypsin 37  
 1 TF104 1  
 PBS 3 0.5μg/Ml trypsin 가 -MEM 가 37 ,  
 CO<sub>2</sub> CPE가 . 70% CPE가  
 - 70 3 . 7,000rpm 5  
 trypsin

1% agarose plaque picking .

60mm3 petridish TF104

10<sup>4</sup> 10<sup>6</sup>

. 1

, 3 PBS

agarose

. agarose

2% agarose

, 42 water bath

water bath

-MEM

1% agarose

.

plate CO2 incubator

3

, CPE가

pasteur pipette

plaque

, 1ml

-MEM

1

가, TF104

가

.

3.

TF104

77

10<sup>6</sup> TCID<sub>50</sub>/ml

5

1.

가 3 2 가 2  
 3 . 2-3-1  
 , 가 1 5  
 , 2 10 . 1  
 , 8 7  
 . 가 2 2  
 가 1 4 6  
 2 1 .  
 3 가 1 가

2-3-1. Virus shedding and diarrhea in goat infected with goat rotavirus

Goat	Virus shedding(day)		Diarrhea(day)		Severity
	Onset*	Duration	Onset*	Duration	
A	1	2	1	1	Intermittent
B	5	10	8	7	Death
C	2	1	-	-	NO
D	2	4	6	2	Intermittent
Control	0	0	0	0	

+ Control \* Days after inoculation

2.

1

2 3  
3  
TF104 77 가  
2-3-2  
77 5  
5 3 가 2 가  
5 3

2-3-2. Pathogenicity of goat rotavirus field isolate and attenuated strain

Virus	Goats inoculated	Diarrhea (No. of goat)	Death (No. of goat)
wild	5	3	2
attenuated*	5	3	0
Control	2	0	0

\* 77 times passaged in TF104 cells



11 dsRNA short, long pattern  
 Reoviridae 가  
 RNA 가 (Estes Cohen, 1989,  
 Taniguchi Urasawa, 1995).  
 SA11, UK, RRV temperative- sensitive mutants (ts mutants)가  
 (Gouvea 1995, Ward 1995).  
 Genogroup 1989 Nakagomi가  
 RNA RNA-RNA hybridization  
 가 .  
 (Nakagomi Nakagomi 1991). Wa, DS- 1, AU- 1 3  
 genogroup , AU- 1 FRV- 1  
 가 (Iizuka 1993, Nakagomi  
 1987, Nakagomi Nakagomi 1989). AU- 1  
 가 가 가 가 (Nakagomi Nakagomi  
 1993). AU- 1 , 가 zoonosis type  
 , , , , ,  
 (Iizuki 1994, Gerna 1990, 1992,  
 Shif 1994, Steele 1993). AU- 1 VP4 가  
 AU- 1 Wa genogroup, bovine genogroup

intergenogroup reassortants (Nakagomi 1992, Urasawa 1993).

VP4 4

VP3 3

VP4 (Estes 1983, Liu 1988). VP4 60

10 12 nm

(Prasad 1988, 1990, Shaw 1993, Yeager 1990). VP4

(Bishop

1991, Galil 1986). VP4가 60KDa VP5 28KDa VP8

(Brandt 1977, Bridger 1987).

가 가 (Birch 1977, Brussow 1992).

VP5 VP8 protease VP4

(Champsaur 1984), protease-enhanced

plaque (Conner 1988). mice piglet virulence

determinant (Eugster 1978). VP4

(Beards 1987),

(Flewett 1976).

VP4 776

, 134 136

1 775 (Carlson 1978).

bovine B223 strain human strain 772 가 VP4가

가 (Blanco 1991). VP4 70% 가 pH 7.0

2 cleavage site

coil and turn . 4 가

VP8 71 204 84 180

VP4 typing (Das 1993). VP4 trypsin

cleavage site arginine 241, 247  
 (Lopez 1985).  
 (Carlson 1978, Connor 1967, De champs 1991, Dharakul 1991,  
 Echeverria 1983, Estes 1979, Flores 1990). VP4가 VP5 VP8  
 가 (Clark 1981), VP5 384 401  
 가  
 (Macow 1988). VP8 79-192 hemagglutinin domain  
 (Bastrado 1980), sialic acid (Fuentes-Panana  
 1995, Mackow 1989, Lee 1995). Rhesus RRV simian SA11 virus VP5  
 VP8 cleavage disulfide bond *in vitro*  
 VP5 VP8 1 disulfide bond 가 ,  
 VP5 segment 1 disulfide bond 가  
 (Flewett 1975).  
 VP7 virion .  
 가 (Champsaur 1984, Conner  
 1991). VP7 6, 7, 8  
 326 (Bellamy Both 1990).  
 50 2 domain (H1 H2)  
 start codon codon  
 (Poruchyndky 1985). VP7 37KDa  
 . domain endoplasmic reticulum  
 51 glutamine .  
 glutamine (Poruchynsky 1985,  
 Stirzaker 1987, Whitfeld 1987), 가  
 (1.5KDa)가 Ile- 9, Thr- 10, Gly- 11  
 (Maass Atkinson 1994). VP7  
 Man8GlcNAc2 single N-linkage 38KDa

(Arias 1982, Kabcenell Atkinson 1985). VP7 G serotype  
6  
hypervariable regions G serotypes .  
NSP4 10 가 (Dyall-smith Holmes 1986,  
Ward 1985) H1, H2, H3 3 175  
(Both 1983). N-linked  
high-mannose oligosaccharide residues 가 (Petrie 1983, Estes  
1989). NSP4 double-layered particle  
(Au  
1989). NSP4 가  
(Ericson 1989, Petrie 1983). VP4 VP7  
oligomerization (Maass Atkinson 1990). NSP4  
가  
(Hoshino 1995), 6 10 114  
135 (Ball 1996).  
VP4, VP7 NSP4  
, Genbank  
. NSP4 RNA-RNA hybridization  
typing(genotyping) .

## 1. Rotavirus RNA

25cm<sup>2</sup> flask TF104 10 MOI(multiplicity of infection)  
 virus CPE가 , PBS 3  
 EDTA- PBS . 10,000rpm 5 cell pellet 100  
 μl PBS . UltraspecTRNA 1 ml 0.2 ml  
 chloroform 가 15 4 5 .  
 15 12,000g .  
 RNA가 0.5 volume isopropanol  
 가 0.05 volume RNA Tack<sup>TM</sup> Resin 30  
 . 1 1ml 75% ethanol 가  
 30 2 .  
 ethanol RNA .  
 DEPC (Biotex, BL- 5610) 1 volume 30  
 1 . RNA가

2. primer

VP4, VP7, NSP4 RT-PCR  
 primer 2- 4- 1 , primer DNASIS program

2-4-1. Primer sequences to genes of goat rotavirus.

Gene	Orientation	Sequence
VP4	F1)	5' - ATGGCTTCACTCATTTA - 3'
	R2)	5' - GAATGCTTGTGAATCATCCC - 3'
VP7	F	5' - GGCTTTAAAAGAGAGAATTT - 3'
	R	5' - GGTCACATCATACAATTCATA - 3'
NSP4	F	5' - TTTTAAAAGTTCTGTTC - 3'
	R	5' - GGTCACATTAAGACCATTCC - 3'

1) F: Forward; 2) R: Reverse

3. cDNA

5.1.1 RNA 10 $\mu$ l Table 3  
 primer 0.7 $\mu$ l 가 100 5 가 .  
 5 $\times$  first strand buffer (250mM Tris-Cl (pH 8.3) 4 $\mu$ l, 10mM  
 dNTP mixtures 1 $\mu$ l, 0.1M dithiothreitol (DTT) 2 $\mu$ l) 가 42 5  
 Superscript reverse transcriptase (Gibco BRL) 1 $\mu$ l 가 42  
 1 , 70 10 .

4. Polymerase chain reaction (PCR)

5.1.3 cDNA 5  $\mu$ l Forward Reverse primer mix 1  
 $\mu$ l, 2.5 mM dNTP mixtures 4  $\mu$ l, 10 $\times$ PCR buffer 5  $\mu$ l, water 31  $\mu$ l, 25mM  
 MgCl<sub>2</sub> 2 $\mu$ l, Taq polymerase (Takara) 2 $\mu$ l 가 . PCR GeneAmp  
 PCR system 9600 (Perkin Elmer) 94 40 , 50 40 , 72 1  
 30 .

5.

PCR 1% agarose gel EtBr

DNA band . 50  $\mu\text{g}/\text{Ml}$  EtBr 1% agarose gel casting  
 5.1.4 well 5 $\mu\text{l}$  100volts 20 30  
 . TBE (Tris-borate 45mM, EDTA 1mM,  
 pH8.0)

6.

Plasmid DNA (0.2 $\mu\text{g}$ ) -21M13 universal primer (1.6 p mol) Big Dye TM  
 Terminator Cycle sequencing Kit (Perkin Elmer, Foster City, CA) 10 $\mu\text{l}$   
 가 model 9700 Thermal Cycler (Perkin Elmer) 96 2  
 denaturation PCR 96 30 , 50 15 , 60 4 , 60 7 25  
 . Pre-hydrated spin columns (Princeton Separations, adelphia, NJ)  
 PCR product mononucleotides loading buffer  
 (deionized formamide/50 mM EDTA, pH 8.0, 5:1, v/v) . 4.5%  
 denaturing polyacrylamide gel (Amresco, OH) loading 90  
 2 가 . loading model  
 377 DNA sequencer (Perkin Elmer) 1,680 volt 9  
 . Sequence Analysis software (version 3.0, Perkin  
 Elmer)

7.

VP4, VP7, NSP4 genotype  
 DNASIS program Genbank Wa  
 (Richardson , 1984: Genbank accession No. L34161), K8 (Genbank accession  
 No. D90260), Mc35 (Genbank accession No. D14032), 1076 (Genbank accession  
 No. M88480), L26 (Genbank accession No. M58292), HAL1166 (Genbank  
 accession No. D14032), Eb (Genbank accession No. L18992),  
 SA11 (Genbank accession No. D16346), RRV (Genbank

accession No. M18736), NCDV (Genbank accession No. M63267), UK (Genbank accession No. M63267), 993/83 (Genbank accession No. D16352), H2 (Genbank accession No. D13397), L338 (Genbank accession No. D13399), MDR13 (Genbank accession No. L10361), OSU (Genbank accession No. X13190), FRV (Genbank accession No. D10971) Lp14 (Genbank accession No. D14032) VP4

VP7 Wa (Genbank accession No. M21843), AU1 (Genbank accession No. D89873), S2 (Genbank accession No. VGXR2S), HCR3 (Genbank accession No. L21666), 116E (Genbank accession No. L14072), L26 (Genbank accession No. M58290), B37 (Genbank accession No. J04334),

Eb (Genbank accession No. U08420), SA11 (Genbank accession No. AH003158), RRV (Genbank accession No. VGSRRN),

NCDV (Genbank accession No. M63266), KK3 (Genbank accession No. D01056), L338 (Genbank accession No. D23549), F123 (Browning 1991), OSU (Genbank accession No. X04613), YM (Genbank accession No. M23194), Gottfried (Genbank accession No. X06759) Ch2 (Genbank accession No. X56784)

NSP4 Wa (Genbank accession No. AF093199), AU-1 (Genbank accession No. D89873), KUN (Genbank accession No. D88829), RRV (Genbank accession No. L41247), NCDV (Genbank accession No. P04511)

## 8. Phylogram

NSP4 type nucleotide sequence NCDV (Genbank accession No. P04511), UK (Genbank accession No. K03384), YM (Genbank accession No. X69486), RRV



(Genbank accession No. L41247), SA11 (Genbank accession No. AF087678),

Wa (Genbank accession No. AF093199), AU-1 (Genbank accession No. D89873), E210 (Genbank accession No. U59107), E201 (Genbank accession No. U59106), S2 (Genbank accession No. U59104), RV3 (Palombo and Bishop 1994), RV4 (Genbank accession No. U59108), RV5 (Genbank accession No. U59103)

FRV-1 (Genbank accession No. D89874) TRICON PROGRAM

phylogram , 가 converseved region

NSP4 VP4 131 148 amino acid

RRV (Genbank accession No. L41247), FRV-1 (Genbank accession No. D89874), AU-1 (Genbank accession No. D89873), M37 (Genbank accession No. U59109), Wa (Genbank accession No. AF093199), NCDV (Genbank accession No. P04511), SA11 (Genbank accession No. AF087678), UK (Genbank accession No. K03384)

## 9. RNA-RNA hybridization

genomic RNA Phenol-chloroform 70mM Tris acetate  
buffer (20mM magnesium acetate, 100mM sodium acetate, 8mM ATP, 0.5mM  
GTP, 2.5mM UTP, 0.5mM s-adenosylmethionine, 0.1% bentonite, [<sup>32</sup>P]GTP, (pH  
8.0)) 250μℓ 42 6 . Phenol-chloroform

lithium chloride ssRNA probe .

RNA-RNA hybridization Fig. 2 . , GRV, FRV,  
RRV ssRNA probes Wa, AU-1,  
FRV-1, FRV64, RRV, R2, NCDV, OSU, Eb dsRNA 100 2

2 ssRNA . probe dsRNA

5mM Tris acetate, 152mM NaCl, 1mM EDTA, 0.1% sodium dodecyl sulfate  
(pH 7.5) buffer 65 16 hybridization ethanol

62.5mM Tris hydrochloride (pH 6.8), 5% (v/v) 2-mercaptoethanol,  
10% (v/v) glycerol, 2% (w/v) sodium dodecyl sulfate, 0.001% (wt/vol) bromophenol

blue sample buffer . Negative-strand genomic RNA  
positive-strand probe hybrids 4% stacking gel 10%  
polyacrylamide gel ethidium bromide gel  
X-Oma AR films (Eastman Kodak Co., Rochester, N.Y.)  
Autoradiographs .

1. VP4  
TF104 RNA VP4  
primer cDNA , PCR . PCR  
pGEMT  
. 2-4-1  
가 2,328 , C486 RRV  
. FRV-1 3 가  
( 2-4-1).  
C486 RRV 776 ( 2-4-2).

GRV	1	ATGGCTTCAC	TCATTIATAG	ACAATTGCTT	ACAAATTCCT	ATACAGTIGA	50
C486	1	*****	*****	***G*****	**T*****A	*C*****A**	50
RRV	1	*****G*	*****	*****	*****A*	***C*****	50
FRV	1	*****TT	*A*****	***G**AT*A	T***C**A*	**GTIACGA*	50
GRV	51	TTIATCTGAT	GAAATACAAG	AAATGGATC	TACAAAACT	CAAAATACAA	100
C486	51	AC*T**A***	****C****	*****	G**T**G***	****CGTT*	100
RRV	51	CC*****	*****	*****	**T**G**G	*****GTC*	100
FRV	51	CA*C****C	**G*TA*C*	*****A*	A*A****CAA	ACT**CGTT*	100
GRV	101	CGATCAACCC	AGGTCATTIC	GCACAAACTG	GTIACGCTCC	AGTGAATTGG	150
C486	101	*CG*T**T**	***A**G***	**G*****AA	A*****T*	**T*****	150
RRV	101	*T**T**T*T	***A**C***	**G*****A*	***T*****	**T**C***	150
FRV	101	*TG*T**T**	***G**G***	*****G*	*A**T**G**	T**IG****	150
GRV	151	GGTCCIGGIG	AAACGAATGA	TICTACTACT	GTCGAACCTG	TATIAGATGG	200
C486	151	**A*****	*****	C**A****A	**T*****A*	*GC*T*****	200
RRV	151	*****	***T*****	*****	**A****G*	**C*T*****	200
FRV	151	**A*A*****	**TIGCC***	****T**IA	**GC****AA	CTC*C*****	200
GRV	200	ACCATATCAG	CCAACCTACGT	TIAACCCACC	AAIAGATTAT	TGGATGTGT	250
C486	200	*****A	****G**T*	***T*****	TG**AG****	*****	250
RRV	200	T**T****A	*****T***	*C**T*****	*G*****	*****C*A*	250
FRV	200	T*****A	**C**T*AC	*C**T***	*G*T*****	*****AA	250
GRV	251	TAGCGCTIAC	GACAGCTGGT	GTTGGTGGG	AAGGAACIAA	TAATACAGAT	300
C486	251	****A**A**	**AC**G***	****A*ATC	***T**C**	C*****A*C	300
RRV	251	****A*****	AG*****A	**A**A**A*	*****	*****C	300
FRV	251	*T*****	T*G**AA**A	AGA**T*CT*	***T**G**	**CG**T**C	300
GRV	301	CGATGGTIAG	CTIACIACCT	AGTIGAACCG	AACGTGACAT	CAGIACTIAG	350
C486	301	A*****	*G**A**A**	*A**A****C	**T**ACAGC	A***TGAGC*	350
RRV	301	*****C***	***A**T**	*****G**T	*****A****	**A***C**	350
FRV	301	A*****T*	**TGIG*GC*	*****A	**T**ACA*A	ATAC*CAA**	350
GRV	351	AACTIACACA	ATATTGGGA	TACAAGAACA	GATGTIAGIA	GCTAACACTT	400
C486	351	***A**T***	T*****C	A****TI**	AGTAAC****	T*A**TGA**	400
RRV	351	**G**T**G	C*****A*	CG*****G**	A**AC*A**	**T**TG***	400
FRV	351	*C*A**GT*	T**GA****C	A*A*T*TC**	AT*GCAG**C	T*A***GA**	400
GRV	401	CACAAACACA	ATGGAAGTTT	ATIGACGTIG	TIAAACTIAC	ACAAAATGGA	450
C486	401	****C*****	C*****	C*G**TC*AA	G***GCAG**	***G****T	450
RRV	401	*C*****	*****A***	***T**C*	*****	*****	450
FRV	401	**AGT**TIC	G*****A***	**ATTIAT*CA	*****TIG**	G*CTG*C***	450
GRV	451	ATATATTCGC	AATACGGACC	ACTACTGICT	ACTCCAAAAC	TTIATGCTGT	500
C486	451	*AT*****A*	**CA**T**	T*****A	**A**G****	*G****GA**	500
RRV	451	*GC*****A*	***A*****	*T***AA***	*****	*C*****C**	500
FRV	451	*CG**CA*T*	****TC**	CT*GT*AA*A	C*G*AT**GT	*A*GCT*GTG	500
GRV	501	AATGAACAT	AATGGIAAAA	TTIATACATA	TAGTGGAGAA	ACGCCGAACG	550
C486	501	G*****	GGA*****	****C**T**	**A*****G	**A*****	550
RRV	501	G*****	*****	*****	**A*****	**T*****T*	550
FRV	501	G*****AGG	C*CAAC*G*G	*G**CTGG**	*CAA***TCG	T*****	550

GRV	551	CAACTACTGG	ATACTATTCA	ACTACAAATT	ATGATTCGGT	GAATATGACA	600
C486	551	*****	T****C**T	**A**T**C*	T***CA**T**	A**C*****	600
SA11	551	TG**C***AA	GT***C***	*****	*****A**	A**C**ACAG	600
FRV1	551	**T*A*GA*A	C**T***IG	***T***CA	****AACAG	C**CG*TT**	600
GRV	601	GCATTTTGIG	ACTTTTATAT	TATACCAAGA	TCAGAAGAAG	CAACATGIAC	650
C486	601	***A*****	*T*****	A**T***TIA	G**C*****	***A***C**	650
SA11	601	*****	*****	*****T***	GA*****T	*****	650
FRV1	601	AGTGACGC**	*A*****T*	G*****GCA*	**GC*GACT*	*T*IG*****	650
GRV	651	AGAATACATT	AACAATGGIC	TACCTCCAAT	CCAGAACACG	CGGAATGIGG	700
NCDV	651	T*****A	**T*****AT	***A*****	A**A**T***	A*A***A***	700
SA11	651	C**G*****	**T**C**GT	*****G**	T***T**A	**A**CA*T*	700
FRV1	651	*C***T**A	**T*****T	***A*****	T***T**A	A*****A*T*	700
GRV	701	TTCCATTAGC	ACTTTCAGCC	AGAAATATTA	TATCACCIAG	AGTTCAGGCG	750
NCDV	701	*A**G*TT*	GA*AGT*T*A	**G*****G	**ATA*A**	**CAC*AC*T	750
SA11	701	*****G**	C*****T	*****A*	*****A***	**C***A***	750
FRV1	701	*A**G**AA	TA**G**T*T	**C*G****	A*GACATA**	**C****AT*	750
GRV	751	AATGAGGACA	TGTTCGTATC	TAAAACGICA	TIATGGAAAG	AGATGCAGIA	800
NCDV	751	***C*A****	*A**G*****	A*****T***	*****	*****A**	800
SA11	751	****A**T*	*C**T**G**	A**C**A***	A*T*****	*****A**	800
FRV1	751	****A****	*A**AA****	G*****T***	*****	*A*****A**	800
GRV	801	CAATAGAGAC	ATTATAATCC	GATTIAAATT	CGCAAACICA	ATTATTAAT	850
NCDV	801	T*****	**AG*G**AA	*****	T**T*****	**C*****	850
SA11	801	*****	**C**TCGAT	*****	*****G**	**C*****	850
FRV1	801	T*****T	**A**C**TA	*****	T**T**T***	**A**C****	850
GRV	851	CTGGTATGAT	GGGTATAAG	TGGICAGAAA	TATCATTIAA	ACCAGCGAAT	900
NCDV	851	*A**C*****	***A*****A	*****G	*G*****	*****T***	900
SA11	851	*C*****GC*	A*****A	*****C*	*T*****	*****A**C	900
FRV1	851	*A*****C*	A*****	***G****	*****	G**CATG**C	900
GRV	901	TATCAATACA	CTTACATGAG	AGATGGGAA	GAAGTAACAG	CACACACAAC	950
NCDV	901	****C****	*A**T*CC**	*****T***	****T**T*	***T**T**	950
SA11	901	*****T*	*G**T*CAC*	*****A**G	**T**T***	*T***G**	950
FRV1	901	*****T*	*G***CA**	*****A**	****G****	***T****	950
GRV	951	ATGTTCIGIA	AATGGAATCA	ATGACTTCAA	CTTIAACGGA	GGATCACIAC	1000
NCDV	951	G*****A***	*****A*	***T**T**	T*A**T**T	*****T***	1000
SA11	951	G**C**A***	**C*****G*	*C**T**T**	T**C**T**G	*****GT***	1000
FRV1	951	*****A**T	****TG**	***T**T**	T*A**T***	**TA*GT***	1000
GRV	1001	CGACGGATT	TGTAATATCA	AGATATGAAG	TAATIAAAGA	AAATICCIAT	1050
NCDV	1001	****T*****	C*****	*A*****	*G*****G**	*****T*	1050
SA11	1001	*A*****	*A*****	*****	*****	G*****T***	1050
FRV1	1001	*T**T*****	**C*****G	***T**C*	*C**A**C**	*****T***	1050
GRV	1051	GIGIATGIG	ATTATIGGA	CGATTCCTCAA	GCTTTIAGGA	ATAIGGTGIA	1100
NCDV	1051	*****A*A*	*C**C*****	*****A***	**A*****A*	*C*****ATG	1100
SA11	1051	**T*****	***C**T**G	T***A***	**C**C****	*C*****T**	1100
FRV1	1051	**A*****A*	*****	T***A***	**A*****	*****A**	1100

GRV	1101	TGIGAGATCG	TIAGCIGCAA	ATTIAAATC	TGIAATIGTG	ACGGGAGGTG	1150
C486	1101	****C*C***	**C**A**CG	*****T**	G*****G	**A*****	1150
RRV	1101	**A**G**A	*****T*	*****G	**T**A**	**T**G***	1150
FRV	1101	**A**G**A	*****T*	***G**TGA	***G**A**C	*GT*****T	1150
GRV	1151	ATTIACAGCTT	TGCATIACCA	GTTGGICAAT	GGCCAGTIAT	GACTGGAGGA	1200
C486	1151	*C**T**T**	**GA*T***	*****A*T*	AT*****G	*****G**T	1200
RRV	1151	***T*****	*****G	*****G	*****A**	*****C***	1200
FRV	1151	C*****T**	**G*****T	**A**CA*TC	AT**G**G**	**G**T**C	1200
GRV	1201	GCAGTTTCCT	TACATICAGC	TGGAGTIACG	TIGTCIACTC	AGTTCACAGA	1250
C486	1201	**T**G**A*	*C*****G	**T**A**T	**A**A**G*	****T*****	1250
RRV	1201	*****G**A*	*G*****G	**T*****G	**A**C**A*	*****G	1250
FRV	1201	*****GA***	**ACA**T**	**T**A**A	C**A**A***	***AT*****	1250
GRV	1251	TTTTGIATCA	TIAAATICTT	IAAGATTIAG	ATTIAGATTA	GCTIGGGAAG	1300
C486	1251	****C*****	*****AC	*G*****G	*****G	T**A**A****	1300
RRV	1251	*****G**A*	**T*****G	***G**C**	G*****C**	A***GT****	1300
FRV	1251	**A*****G	*****A*	*GC***C**	***C*****G	**G***ACC*	1300
GRV	1301	AACCATCGTT	TGCGATIACT	AGAACIAGAT	TCAGTAGACT	ATATGGACTA	1350
C486	1301	****GC***	CT*A**CTA	C**G**C**G	*T***G**T*	G*****T	1350
RRV	1301	*G*****A**	CT***C**C	*****G	*TC**C**T*	G*****GT**	1350
FRV	1301	*****A**	*T**T**CT*G	C*****A	*C**G**CA*	***C**T**	1350
GRV	1351	CCAGCTGCTA	ATCCAAACAA	CGGAAAGAG	TGTITGGAAG	TAGCAGGTAG	1400
C486	1351	*****G**A*	*A**G**T**	TTCAC***A	*A**A***GA	****T**G**	1400
RRV	1351	**T**A**T	*C**C**T**	T**A*****	*A**A***G	*G**T**C**	1400
FRV	1351	*****TA*	*****T**	TAACGC**A	*A**A***GA	****T*****	1400
GRV	1401	ATICTCATIA	AIATCGCTAG	TICCAICTIA	CGATGATAC	CAGACACCAA	1450
C486	1401	***T*****	*****A**C*	*A**G**A**	T*****T	*****G	1450
RRV	1401	*C*****C**	*****AT*G*	*A*****G	T**C*****	*****G	1450
FRV	1401	*****C*C	*****A***	*A**A**A**	T*****C**T	**A**G****	1450
GRV	1451	TAGCCAATC	AGTGACAGTT	AGACAAGATT	TAGAGCGCA	GTTGGGTGAG	1500
C486	1451	**ATA**T**	***C**T**A	C*****G	***A**A**	A**A**A**A	1500
RRV	1451	**A**T**T**	***T*****C	*****G	***A**A**	*****A	1500
FRV	1451	*C**T**T**	***T**C**G	*****G	***A**A**	A**A*****	1500
GRV	1501	CTIAGAGAAG	AGITTAATGC	TTIATCGCAA	GAAATAGCGA	TGICCCAGTT	1550
C486	1501	**A*****T*	*A*****CAA	*****A***	C****C**T*	***A**AC*	1550
RRV	1501	*****G**A*	*A**C**C**	*C**C**A***	**G*****G	***G***C*	1550
FRV	1501	T*A*****	*A**C**T*	G**G**A***	*****TG	*T****AC*	1550
GRV	1551	GATCGATTIG	GCGTIGCTCC	CATTAGATAT	GTTTCAATG	TTTICIGGIA	1600
C486	1551	***A**C**T	*****A**A*	*CG***C**	***C*****	*****A**G*	1600
RRV	1551	T**TT*****	**A**A**T*	***G*****	*****G***	*****G	1600
FRV	1551	T**A**CC*A	**AACA**A*	*GC**T****	***C*****	**C*****A*	1600
GRV	1601	TIAAAAGCAC	TATAGACGCA	GCTIAAATCAA	TGGCTACTIA	TGIAATGAAA	1650
C486	1601	****G**T**	*A**T*****	**C**C**T*	***G**G**	*****G	1650
RRV	1601	***G*****	C*****T**	*****G	*****G	*****G	1650
FRV	1601	*A**TCA**	GG***G***	*IA*****T*	**A**G**	C**G*****	1650

GRV	1651	AAATTIAAGA	AATCAGGTTT	AGCTAGTTCA	GIATCIACAT	TGACAGATTC	1700
C486	1651	**A****A*	*C***A**C*	C***AC***	**G**A**GC	*C**T*****	1700
RRV	1651	*****	*****	*****AC**T	*****	*A****C**	1700
FRV	1651	*G****A*	C***A****	**A*ACG*C	A****GAT*	*A**AGCAA	1700
GRV	1701	ATTATCCGAC	GCAGCCICTT	CAATATCAAG	AGGAGCATCC	ATTCGTTACG	1750
C486	1701	**G**T**T	*****A**A*	***T**T**	*A*T*****G	G**A*A****	1750
RRV	1701	*C*G*****	*****T****	***T*****	*****T	*****	1750
FRV	1701	TA*G**G**A	**G**A**A*	*TG**AG*TT	GACGT**GTA	*GATCGATT*	1750
GRV	1751	TGGGATCATC	AGTTTCAGCG	TGGACAGATG	TCTCAACACA	AGTTACTGAC	1800
C486	1751	*TA*T***A*	T*CA*****T	*****C**A*	*A**T*ACAT	TACAT**A**T	1800
RRV	1751	*T*****	**CA*****A	*****	*****	*A*C****T	1800
FRV	1751	GCAGTITA*	*T*GC**AGA	GCT*G**T*T	CA*TGCA*GT	*AG*GA****	1800
GRV	1801	ATTTCCTCGT	CIGTTAGTAC	GATTTCAACG	CAGACTTCGA	CTIATIAGTAG	1850
C486	1801	**AATGT*A	*AACG**CT*	**C**T**A	*****A**A*	*A****C**	1850
RRV	1801	G*****A*	***C**T*	**C**C**A	*****A*	*****	1850
FRV	1801	T*AAGG**TA	TGCAGGACGT	ATCAA**CAA	GTGT*AAATG	TG*G***A*A	1850
GRV	1851	AAGGTTGAGA	CTAAAAGAAA	TGGCTACTCA	AACAGAAGGA	ATGAATTTTG	1900
C486	1851	*****A**	*****	***G*****	**G**C**T	*****	1900
RRV	1851	*C**C*AC**	*****	*****G**	*****G	*****C*	1900
FRV	1851	TTT*AGATTG	AA*G*GTTC*	C*A*GCAAAC	TGATACTTT*	*GCTT*GA**	1900
GRV	1901	ATGATATATC	CGCCGACGTA	TAAAAACTIA	AAATTGATAG	ATCTACTCAG	1950
C486	1901	*****	A**A*****	C*C**G****	*****A	**A**C***	1950
RRV	1901	*****	T**T*****	**C**G****	*****C*	**C*****A	1950
FRV	1901	*CATCTCTG*	A**T*I*TTG	AAG*CG*AAC	T*GACA*ATC	GA*GCAAATT	1950
GRV	1951	ATTTCICAC	ACACGCIACC	AGATATGTIC	ACTGAGGCTT	CGGAAAAGTT	2000
C486	1951	T*AAA*A**A	*T**AT*G**	G**A**A**A	*****	*A*****	2000
RRV	1951	**A*****A	***AT****	*****A***	*****A****	*A**G****	2000
FRV	1951	TCACAA*AAA	CG*T**C*GA	IAT**A*CT	GAGTCAT**G	AAA*GTTA*	2000
GRV	2001	TATTCCTAAT	AGAGCATATA	GAGTAAATAA	TAATGATGAA	GIGTTTGAAG	2050
C486	2001	**A**A***	*****C**C*	*T****T**	AGA*****	**C*A**C*	2050
RRV	2001	*****	*****C**C*	*****T**	*****	**C*****	2050
FRV	2001	ACCGAAA*GA	TCGTACAGA*	T**TGATG*	GG**ACC*C*	T*CGAAACT*	2050
GRV	2051	CTGGAACAGA	TGGTAGGTTT	TTTGCTIATC	GCGTTGAAAC	ATTCGAAGAA	2100
C486	2051	**A*T**T**	*****AA*A*	**C*****CA	AA*****C*	CA*TTTGA*G	2100
RRV	2051	*G*****	**A**A**A*	*****C****	*T*****	G*****T***	2100
FRV	2051	GAATTGAC*G	AACGTTT*A*	GC*TACA*AG	T**A*AC*TT	TAAT**ATT	2100
GRV	2101	ATACCGTICG	ATGTGCAGAA	ATTTCGGAT	TIAGTACTG	ATCTCCAGT	2150
C486	2101	*G*TTCCATT	CGA**T*C**	***C**T**C	*****	*C**A*****	2150
RRV	2101	**T**A**T*	*****A**	G*****A***	C****A****	*C*****G**	2150
FRV	2101	CCGTTTGATA	TG*AA*GATT	IAA*AAATTA	A**ACAGACT	CACCAGTTI*	2150
GRV	2151	GATCTCAGCT	ATAATAGATT	TCAAGACATT	GAAGAATTTG	AATGATAATT	2200
C486	2151	T**A**C**A	*****T**C*	*T**A**TC*	T*****C*A	*****	2200
RRV	2151	C*****C	**T*****C*	*T*****C*	C*****C*A	**C**C****	2200
FRV	2151	ATCAG**ATA	**GACTTTA	AG*C*TT*AA	**CTIAAAC	G**A**T**G	2200

GRV	2201	ATGGIATTAG	TAAACAACAA	GCATTIAATT	TGTIAAGATC	CGATCCAAGG	2250
C486	2201	*C**A**A**	C*C*****	***C*A***C	CIAAG*****	T*****GC*A	2250
RRV	2201	*****	**GG*****	*****C	**C*****	*****A	2250
FRV	2201	GAATA*CA*A	G*****GCC	ATGGAACIA*	*ACATTC*AA	TCCAAAG*CA	2250
GRV	2251	GIATTIACGTG	AGTTCATIAA	TCAAGATAAT	CCAATAATAC	GGAACAGGAT	2300
C486	2251	*****	*A**T*****	***G*****	*****	*A**T*G***	2300
RRV	2251	*****	*A**T**C**	*****C***	*****T*	*T*****A**	2300
FRV	2251	T**AA*GAGT	TIA*A*A***	*A*TA**CCA	ATA**T*G*A	AT*GA*TTGA	2300
GRV	2301	TGAACAATIA	ATAATGCAAT	GTAGATTG			
C486	2301	A***AGT**G	*****	**CGC***			
RRV	2301	*****C***	*****G*	*****C**			
FRV	2301	AA*TTT*A**	TCGCA*IGTA	*GTTG			

2-4-1. Comparison of VP4 nucleotide sequences between GRV and other strains.



GRV	1	NASLIYRQLL	TNSYTVDLSD	EIQEIGSTKT	QNTIINPGPF	AQTGYAPVNW	50
C486	1	*****	*****I**	*****	**V**V**	***N**S**	50
RRV	1	*****	*****	*****	**V**I**	*****	50
FRV	1	*****	S**VINI**	*VN**IK**	T*V**V**	*****D*	50
GRV	51	GPGETNDSTT	VEPVLDGPYQ	FTTFNPPIDY	WMLLAPTIAG	VVVEGINNID	100
C486	51	*****	*****	*****VS*	*****N**	**DQ*****	100
RRV	51	*****	*****	**S**V**	*****A**	*****	100
FRV	51	*H**LP**L	*Q*T*****	**SL*L*V**	***I**REG	RVA***T**	100
GRV	101	RWLATILVEP	NVISVIRIYT	IFGIQEIVV	ANISQIQVKF	IDVVKTIQNG	150
C486	101	*****IK*	**QQ*E****	I**Q*V*VI*	S*D**K***	V*LS*Q**D*	150
RRV	101	*****	***E**S**	I**T***TI	*YA*****	VKL**Q****	150
FRV	101	**I*CV****	**QNTQ*Q*V	*D*QNV*LQV	S*D*S*S***	VLFI*I**D*	150
GRV	151	IYSQYGPLLS	TPKLYAVMKH	NGKIYIYSGE	TPNATIGYYS	TINYDSVNMT	200
C486	151	N***H*****	*****	G*****N**	*****	***F*T****	200
RRV	151	S*****Q*	*****G****	*****N**	***V**K***	*****	200
FRV	151	T*T**S**ST	PH**CSW**R	DNRV*V*Q*S	S***SES**L	*I*N*NSNVS	200
GRV	200	AFCDFYIIPR	SEEATCIEYI	MNGLPPIQNT	RNVVPLALSA	RNIISPRVQA	250
C486	200	*Y*****L	AQ**K*****	N*****	**I**VSIVS	***VYT*A*P	250
RRV	200	*****	E**S*****	N*****	**I*****	***I**A**	250
FRV	200	SDAEF*L**Q	*QT*N**Q**	N*****	*I**VNIAS	*Q*KDI*A*M	250
GRV	251	NEDIVVSKTS	LWKEMQYNRD	IIIRFKFANS	IIKSGGLGYK	VSEISFKPAN	300
C486	251	*Q*****	*****	*V*****	*****	***V*****	300
RRV	251	*Y*****	*****	*I*****S*	*V*****	*****	300
FRV	251	****I****	*****	*****	*****	*****N*	300
GRV	301	YQYTYMRDGE	EVIAHITCSV	NGMNDNFENG	GSLPTDFVIS	RYEVIKENSY	350
C486	301	****T****	*****	**I***Y**	*****	K*****F	350
RRV	301	****T****	D*****	*****	*****I**	*****	350
FRV	301	****T****	*****	**V***Y**	*I*****A**	*I*****	350
GRV	351	VYVDYWDSDQ	AFRNMVYVRS	LAANLNSVMC	TGGDYSEALP	VGQMPVMTGG	400
C486	351	**I*****	*****	**D*****	*****I*	**NYP*****	400
RRV	351	*****	*****	*****I*	*****	*****	400
FRV	351	*****	*****	*****J*V*	S**S*****	**NH**S**	400
GRV	401	AVSLHSAGVT	LSIQFIDFVS	INSLRFRFRL	AVEEPSFAIT	RIRVSRLYGL	450
C486	401	*****	*****	*****	S***P*S*L	****G****	450
RRV	401	*****	*****	F*****	T*****S**	***GC****	450
FRV	401	**T*T****	***Y**Y**	*****	**S***S*S	***N*GI**	450
GRV	451	PAANPNNGKE	CCEVAGRESL	ISLVPNSDDY	QIPIANSVTV	RQDLERQLGE	500
C486	451	**K**SQ*	YY*I*****	*****	***I*****	*****	500
RRV	451	**Y*****	YY*****I**	**I*****	***T*****	*****	500
FRV	451	**V***NA*	YYEI*****	*****I***	*****	*****	500
GRV	501	LREEFNALSQ	EIAMSQLIDL	ALLPLDMFSM	FSGIKSTIDA	AKSMATNVMK	550
C486	501	**D***N***	Q*****	*****	*****	*****	550
RRV	501	*****	*****Y*	*****	*****	*****S**	550
FRV	501	*****S**	**V*****	*I*****	*****VE*	V**T*****	550

GRV	551	KFKKSQLASS	VSTLIDSLSD	AASSIRGAS	IRSVGSSVSA	WIDVSTQVID	600
C486	551	R***S**N*	*****	*****S**	V**S*IA**	**E**NIS**	600
RRV	551	T***G**N*	*****	*****	*****A**	*****I**	600
FRV	551	**T*S**NA	I*D**SNV*E	***VRLTSV	RSIGIVLPR	ARVSLQVSD*	600
GRV	601	ISSSVSTIST	QTSTLSRRLR	LKEMATQTEG	NFDDISAAV	LKTKIDRSTQ	650
C486	601	*NVIT*S***	*****	*****D*	*****	*****K***	650
RRV	601	V*****	*****	*****	*****	*****	650
FRV	601	LR*MQDVSTQ	VSNVSRNLRL	KEFTIQDTIL	SFDDISA*VL	KTKLDKSTIQI	650
GRV	651	ISPHILPDIV	TEASEKFIPN	RAYRVINNDE	VFEACTDGRF	FAYRVETEFEE	700
C486	651	LNynt**E**	*****	*****KD**	*L**S**GKY	**K***ILK	700
RRV	651	*****	*****	*****	*****	*****D*	700
FRV	651	SQQIMPDI*A	ESSEKFI PKR	SYRIVDEDTA	FETIGDIGIFY	AYKVDTFN*I	700
GRV	701	IPFDVQKFAD	LVTDSPVISA	IIDFKILKNL	NDNYGISKQQ	AFNLLRSDPR	750
C486	701	RFHSVY****	*****	*****	*****R**	*I*****	750
RRV	701	*****	*****	*****	*****R**	*****	750
FRV	701	PFDMERFNKL	ITDSPVLSAI	*DFKILKNLN	DNYGITK**A	MEL*HSNPKT	750
GRV	751	VLREFINQDN	PIIRNRIEQL	IMQCRL			800
C486	751	*****	*****S*	*****			800
RRV	751	*****	*****	*****			800
FRV	751	LKEFINNNP	I*RNRIENLI	SQCRL			800

2-4-2. Comparison of VP4 amino acid sequences between GRV and other strains.

2.	VP4	p type					
			VP4 type		nucleotide		
amino acid		NCDV, UK, B223, 993/83,		OSU, MDR13,			
RRV,		AU-1, SA11, 1076, K8, 69M, Mc35, HAL1166,					
L338,	Eb,	Lp14		VP4	sequence		
homology		type 1 NCDV	71/75%, type2		SA11		
79/88%, type3		RRV가 83/91%		, type 4	L26		
72/71%, type 5	UK	72/76%, type 6		1076	72/72%,		
type 7	OSU	75/80%, type 9	Wa	72/71%, type 9			
K8	70/69%, type 10	69M	76/85%, type 11		B223		

60/81%, type 12                      B2            75/81%, type 13                      MDR13  
 75/78%, type 14                      Mc35            70/70%, type 15                      Lp14            76/82%,  
 type 16                      Eb            71/76%, type 17                      993/83            67/63%, type 18  
                     HAL1166            70/71%, type 19                      L338            75/78%  
 .                      3                      RRV            83/91%  
                     3            가    ( 2-4-2).

2-4-2. Sequence homology between VP4 of goat rotavirus and those of other rotavirus strains

Strains	VP4 genotype	P serotype	% sequence homology	
			Nucleotide	Amino acid
NCDV	1	P6	71	75
SA11	2	P6	79	88
RRV	3	P5	83	91
L26	4		72	71
UK	5	P7	72	76
1076	6	P2A	72	72
OSU	7	P9	75	80
Wa	8	P1A	72	71
K8	9	P3A	70	69
69M	10	P4	76	85
B223	11	P8	60	60
H2	12		75	81
MDR13	13		75	78
Mc35	14	P3B	70	70
Lp14	15		76	82
Eb	16	P10	71	76
993/83	17		67	63
HAL1166	18	P11	70	71
L338	19		75	78

3. VP7  
 TF104 RNA VP7  
 primer cDNA , PCR . PCR  
 pGEMT  
 Fig. 7 . Fig. 7 가  
 978 , NCDV SA11,  
 AU-1 ( 2-4-3).  
 NCDV, SA11 AU-1 326  
 ( 2-4-4)..

GRV	1	ATGIATGGTA	TTGAATATAC	CACAATICTA	ACCCITTTGA	TATCATICAT	50
NCDV	1	*****	*****	*****	*T*T*C****	C***GA*T*C	50
SA11	1	*****	*****	***G*****	**T**C***	***GA*T**	50
AU-1	1	*****	*****	***G*T**	**T*****	***G*T**	50
GRV	51	TTTATGAAC	TATATATTAA	AATCTTAAAC	CAGAGIGATG	GACTICATIA	100
NCDV	51	A*****T	*****C****	***AA****	G***A*****	***AT**A*	100
SA11	51	*C**C*A**T	**C**C*T*	***A*****	T***A*A***	***GT**A*	100
AU-1	51	A*****T	**CG**C*C*	***A*****	T***A*A***	***T****	100
GRV	101	TTTACAGATT	TCFTTTCATT	ATIAGTIGTTT	TGTCACCATT	ACTGAAAGCT	150
NCDV	101	*****	**GC*T**A	G***GA*C*	**G*CA*CA*	*A**T**G	150
SA11	101	***T****	G*****A	**T**GA*A*	*****	T**C**G**A	150
AU-1	101	*****	*****A**	*****A*AC	*****	C*T**T**A	150
GRV	151	CAAAATIACG	GGATTAATCT	GCCAATIACT	GGTTC AATGG	ATACIGCAIA	200
NCDV	151	*****C**T*	*AG*A**T*	*****A	*****	*****G**	200
SA11	151	*****T*	*T*****	T*****C**A	**C**C****	*C*****	200
AU-1	151	*****T*	*A**A*****	T**G*****	*A*****	*C**AC****	200
GRV	200	CGCTAATICT	ACACAAGAAG	AAACTTTCCT	CACATCAACT	CIGIGCTIAT	250
NCDV	200	T**AG*C***	*****AGT*	*GC*A**T*	G*****C	**T**T**G*	250
SA11	200	*****A	**G*****	***A*****	**T**T**A	**T**CC***	250
AU-1	200	TA*G**C*A	***G**G*	**GTA*****	AA*T**G***	T*A**T**G*	250
GRV	251	ATIATCCAAC	TGAAGCGCGG	ACTGAAATAA	ATGATAATTC	GIGGAAAGAT	300
NCDV	251	*****TGT	**G**AT*A	*AC*****G	C*****CCGA	A*****	300
SA11	251	*****G**	**G**T**	*****	*C*****	A*****C	300
AU-1	251	***C*****	*****A**A	**A*****	*****	A***G***	300
GRV	301	ACACTCICTC	AACIGTTTTT	GACTAAGGGA	TGGCCCACCG	GATCCGTTIA	350
NCDV	301	**CT*A**A*	**T***C**	***A**A***	*****A**A*	***A**G**	350
SA11	301	*****G**A*	***A***C*	T**G**A**G	*****A*T*	*****A**	350
AU-1	301	***T****	*G*A****G	T***A***	*****A**A*	*****A****	350
GRV	351	TTTTAAAGAA	TACGCTGATA	TCGCTICTTT	TTCAGICGAT	CCGCAATIGT	400
NCDV	351	CC*****	**T*****	*A**GG*C**	*****G**A	**A**G**A*	400
SA11	351	*****	**TA**A*C*	*T**A**G**	**T**T***	***C****	400
AU-1	351	*****T	**TA**A***	*T**C**G**	*****T***	**A*****A*	400
GRV	401	ACIGCGACTA	TAATGIAGTA	CIAATGAAAT	ATGATGCAAC	TTIGCAATIG	450
NCDV	401	G*GATT*IA*	***T***T	T*****	***ICT**	ACAAG**C*A	450
SA11	401	*T**T**T**	**C*****	*****	***C**G**	G*****	450
AU-1	401	***T**T**	***T**C**	T*****	*C**C**T**	AC*****C**	450
GRV	451	GACATGICCG	AACTIAGCTGA	TCIAATATIG	AATGAATGGC	TGTGCAATCC	500
NCDV	451	**T*****T*	**T**G**C**	***T*****	**C*****	*****	500
SA11	451	**T*****A*	***T**G**	*****A	**C*****T	***T****	500
AU-1	451	*****	*****A**	**GT**C*T	***C**T	*A**T****	500
GRV	501	AATGGACATT	ACTCTATATT	ATTATCAACA	AACAGATGAA	GCTAACAAAT	550
NCDV	501	*****A	**G*****	*****G**	C**T*****	**A**T****	550
SA11	501	*****T**	*****G****	*****G**	**T**C***	**G**T****	550
AU-1	501	T*****T**	**T**G****	*****	**T*****G	**A**T****	550

GRV	551	GGATTICTIAT	GGGATCTTCG	TGCACAATAA	AAGTAIGTCC	GCTTAATACA	600
NCDV	551	****A**A*C	***C*****T	*****GC*T*	***G*****	AT*A*****	600
SA11	551	****A**A**	***C**A**A	**T****T*	*****	A*****	600
AU-1	551	*****A**	*****A**T	**T**C****	*G*****	A**A*****	600
GRV	601	ACGACTCTTG	GAATCGGTG	TTAACTACT	GATGCGACAA	CATTCGAAGA	650
NCDV	601	CAA**A****	*T**T**A**	*C***TA***	A**C*AGAC*	*G**T***AC	650
SA11	601	CAA*****	***T**A**	C**G**A***	***T****	*T**I****	650
AU-1	601	CAA**AT*A*	***T**G**	CC*****	**A*A*AC*	*G*****	650
GRV	651	AGTGCTIACA	GCTGAGAAAT	TGTAATTAC	TGACGCGTC	GATGGAGTGA	700
NCDV	651	*****G***	ATC*****G*	*A**G*****	A**T**T**A	*****T**C*	700
SA11	651	*****G***	*****A**G*	*****	*****G**T	*****C**T*	700
AU-1	651	*****A***	*****	*A**G*****	*****T**A	*****G**C*	700
GRV	701	ATCATAAAT	TGACGTTACA	ACTGCTACTT	GCACIATTAG	AAATIGIAAA	750
NCDV	701	****C***T*	AAA**C***	**C*A***G*	****C**AC*	C**C*****	750
SA11	701	*****G**	G**T**C***	**A**A**G*	*T*****	**C*****G	750
AU-1	701	*****T*	GA***G**G	**AAAC****	*T**A**C**	*****	750
GRV	751	AAATIGGGAC	CAAGAGAAA	CGTGGCAGTA	ATTCAAGTTG	GAGGTICTGA	800
NCDV	751	**G**A****	***G**G**	***Q*****C	**A**C**A**	*C**CG*AA*	800
SA11	751	*****	*****	***A**C**T	**A*****	*T*****	800
AU-1	751	****A****	***G*****	**A*****T	**A**G****	*T**C**A**	800
GRV	801	CGTICTIGAT	ATAACAGCTG	ATCCAACIAC	AGCACCCGAG	ACTGAAAGAA	850
NCDV	801	T**T*A**C	**C*****	*****A**	TA***A***	**A**G****	850
SA11	801	*A**C**C***	***T****	*****	T*****A***	**A**C**G*	850
AU-1	801	TA*A*****C	*****	*****G**	**G**A**A	**A*****	850
GRV	851	TGATGCGIAT	TAATIGGAAA	AAATGGTGGC	ACGTATTCIA	CACAGIAGTT	900
NCDV	851	*****A**	A*****	*****	*A**G**T**	***G*****G	900
SA11	851	*****A**	***C*****	*****	*A**T**T**	T**T*****A	900
AU-1	851	*****AG*	G*****G	*****	*A*****T**	T**A*****	900
GRV	901	GACTATATAA	ATCAAATAAT	ACAAGCAATG	TCCAAAAGAT	CACGATCACT	950
NCDV	901	**T**CG*C*	***G*****	T**GA*****	*****	*TA***G**	950
SA11	901	*****C**G	***G*****	***T***	*****	**A*****	950
AU-1	901	****CGTG*	*****TG*	G*****C***	*****	*GA*****T*	950
GRV	951	GAACTICGCT	GCATICTIAC	ATAGAGTT			1000
NCDV	951	T**T**GT*G	**G*****	*****G			1000
SA11	951	A**T**A**A	*****T**T*	*C*****G			1000
AU-1	951	A**A*****	*****T****	*C**GA*A			1000

2-4-3. Comparison of VP7 nucleotide sequence between GRV and other strains.

GRV	1	MYGIEYTTIL	TLISFILLN	YILKSLTRVM	DFIIVRFLFI	IVVLSPLLKA	50
NCDV	1	*****	*F*T*IT**	*****I**N*	*Y**Y***L*	V*I*ATIIN*	50
SA11	1	*****V*	*****I****	*****I*	*C**Y*I***	**I***F*R*	50
AU-1	1	*****V*	*F**V****	*V*****I*	***Y***L*	*****F*N*	50
GRV	51	QNYGINLPIT	GSMDIAYANS	TQEETHLTST	LSLYPTEAA	TEINDNSVKD	100
NCDV	51	***V*****	*****I*	**S*D*****	*C***V**S	N**A*TEV**	100
SA11	51	*****	*****P*I**	*R**V*****	*C*****	*****	100
AU-1	51	*****	*****	*****	*C*****	*****	100
GRV	101	TLSQLFLTKG	WPTGSVYFKE	YAKIASFSVD	PQLYCDYNVV	LMKYDATLQL	150
NCDV	101	*****	*****I*	**D*A**E	*****I*	*****S*QE*	150
SA11	101	*****	*****	*TN*****	*****	*****	150
AU-1	101	*****C**	*****I**D	*TN*****	*****I*	*****	150
GRV	151	DNSELADLIL	NEVLCNPMDI	TLYYYQTDE	ANKVISMGS	CIHKVCPINT	200
NCDV	151	*****	*****	*****	*****I**	**V*****	200
SA11	151	*****	*****	*****	*****	*****	200
AU-1	151	*****I*	*****	*****	*****	*****	200
GRV	200	QTLGIGCLTT	DATIFEEVAT	AEKLVITDVV	DGVNHKLDVT	TATCTIRNCK	250
NCDV	200	*****I*	NPD**I**	N*****	*****N**	*****	250
SA11	200	*****	*****	*****	*****	*****	250
AU-1	200	*****	*TN*****	*****	*****N**	*N*****	250
GRV	251	KLGPRENVAV	IQVGSVDVLD	ITADPTIAPQ	TERMRINWK	KVVQVFYIVV	300
NCDV	251	*****	*****AN**	*****I**	*****	*****	300
SA11	251	*****	*****I**	*****	*****	*****	300
AU-1	251	*****	*****I**	*****	**R**V***	*****I*	300
GRV	301	KYINQIIQAM	SKRSRSLNSA	AFYYRV			350
NCDV	301	D*V*****I*	*****S	*****			350
SA11	301	D*VD***V*	*****	*****			350
AU-1	301	D*V***V***	*****	*****I			350

2-4-4. Comparison of VP7 amino acid sequence between GRV and other strains



4. VP7 g type

	VP7 type		nucleotide
amino acid	NCDV, KK3,	OSU, YM,	RRV,
SA11,	Wa, Gottfried, HCR3,	L338, F123,	Eb,
Ch2	sequence homology		( 2- 4- 3).
type 1	Wa 75/81%, type 2	S2 75/75%, type 3	
Eb	77/89%, HCR3 85/95%, RRV	76/81%, SA11 83/95%, type	
4	Gottfried 75/78%, type 5	OSU 78/85%, type 6	
NCDV	77/85%, type 7	Ch2 67/59%, type 8	B37
75/80%, type 9	116E 77/85%, type 10	KK3	
77/83%, type 11	YM 78/88%, type 12	L26 75/81%,	
type 13	L338 77/82%, type 14	F123 83/87%	
.	3	Eb, HCR3, RRV, SA11 76 85/81 95%	가
		3	가

2-4-3. Sequence homology between VP7 gene of goat rotavirus and those of other rotavirus strains

Strains	G type	% sequence identity with other rotaviruses	
		Nucleotide	Amino acid
Wa	1	75	81
S2	2	75	75
Eb	3	77	89
HCR3	3	85	95
RRV	3	76	81
SA11	3	83	95
Gottfried	4	75	78
OSU	5	78	85
NCDV	6	77	85
Ch2	7	67	59
B37	8	75	80
116E	9	77	85
KK3	10	77	83
YM	11	78	88
L26	12	75	81
L338	13	77	82
F123	14	83	87

5. NSP4  
TF104 RNA NSP4  
primer cDNA , PCR . PCR  
pGEMT  
. 2-4-5  
가 525 , NCDV, RRV, FRV,  
Wa, AU-1, KUN .  
NCDV, RRV, FRV, Wa, AU-1, KUN 175  
( 2-4-6).

GRV	1	ATGGAAAAGC	TTACCGACCT	CAATTACACA	TTGAGIGTAA	TCACICTTAT	50
NCDV	1	*****	*****	**C**T**	*C*****	*****A**	50
RRV	1	*****	*****	**C*****	*****G	*****C**	50
FRV1	1	*****	*****	*****	*****	*****	50
VA	1	****T***	**C*****	**C*****	*****	****TCA**	50
AU-1	1	*****	*****	**C*****	*****	*****	50
KUN	1	*****T	*****	*****G	*****	****T*A**	50
GRV	51	GAATGATACG	TTGCACTACTA	TAATGGAGGA	TCCTGGAATG	GCGTATTTC	100
NCDV	51	**CAGC**A	*****C**G*	**C**C*****	**A**G***	*****	100
RRV	51	*****T	**A*****C*	*****	*****	*****	100
FRV1	51	*****C**	*****C*	*****	*****	*****C****	100
VA	51	*****C**A	*****T**	****TC*A**	*****	*****	100
AU-1	51	*****C**	*****C*	*****	*****	*****C****	100
KUN	51	****AG***A	**A*****A*	**C*A*****	**A*****	*****	100
GRV	101	CATACATAGC	TTCTGTCTA	ACIGIACIAT	TCACATTACA	TAAAGCTTCG	150
NCDV	101	*T**T*****	A*****C***	**A**TT*G*	***G**G**	C****A**T	150
RRV	101	*****T**	*****C***	*****	*T*****	**G**C***	150
FRV1	101	***T**T**	*****C***	*****G*	*T**G*****	*****A	150
VA	101	T**T**T**	*****	**A**TT*G*	*****	*****A	150
AU-1	101	***T**T**	*****C***	*****G*	*T**G*****	*****A	150
KUN	101	*T**T**T**	A*****C**G	**A**TT*G*	*****	C****G**A	150
GRV	151	ATICCAACCA	TGAAAATIGC	CCTIAAAGC	TCAAATGCT	CATATAAAGT	200
NCDV	151	*****A	*****	A**G**C**	**C*****T*	*****	200
RRV	151	G*****	***G*****	T*****	***C**T**	*****	200
FRV1	151	*****T*	***G*****	T***C***A	***C***	*****	200
VA	151	*****	*****A**	AT*G***A	*****T*	*****	200
AU-1	151	*****T*	***G*****	TC**G***A	***GC****	*****	200
KUN	151	*****G*	*****A**	AT*G**G**	*****	*G*****	200
GRV	201	AAICAAATAT	TGIATCGIAT	CGATTTTIAA	CACICIGTIG	AAACIGGCTG	250
NCDV	201	GG*G**G**	*****T**GA	*****C**	T**GT*****	**T***A*	250
RRV	201	*****C	**C**T**G*	*A*****	*****A**	*****	250
FRV1	201	*****G**	*****T***	*A**C*****	***T*AC*A	*****	250
VA	201	G**T*****	*****A**CA	*****A****	T****T**A	**T*****	250
AU-1	201	*****G**	*****T***	*A**C*****	***T*AC*A	*****	250
KUN	201	*G*A**G**	**C**T***A	***CC****	T**AT**A**	**T**A**A*	250
GRV	251	GATATAAAGA	ACAAATCACT	ACTAAAGACG	AAATTGAGAA	GCAGATGGAC	300
NCDV	251	*T*****	**G**T***	*****T*	*G**A**A**	***A*****	300
RRV	251	*****	*****T**	*****T*	*****A*G	***A*****	300
FRV1	251	*G*****	**G**T***	*****	*****A**	***A*****	300
VA	251	*****	G**GG*T**	*A*****	*****C*	A*****	300
AU-1	251	*G*****	**G**T***	*****	*****A**	***A*****	300
KUN	251	*T**C*****	**G**T***	*****T*	***A**A**	A**A*****	300
GRV	301	AGAGTTGIAA	AAGAGATGAG	ACGTCAGCTG	GAAATGATCG	ATAAACTAAC	350
NCDV	301	**C**A**T*	*****	*****A	*****T*	***GT*G**	350
RRV	301	*****	***A*****	*****	*****T*	***C*****	350
FRV1	301	**C*****	***A*****	*****	*****T*	***C*****	350
VA	301	**A***G*	*****	*****	*G*****T*	*****	350
AU-1	301	*****	***A*****	*****	*****T*	***C*****	350
KUN	301	****C**T*	***A*****	*****AT*A	*G*****T*	*****	350

GRV	351	TACTAGAGAA	ATTGAACAGG	TIGAGTTACT	TAAGCGAATC	CATGATATGC	400
NCDV	351	***AC*T***	*****G****	*G**A**G**	A**A**C**T	**C****AAT	400
RRV	351	C*****G	*****G**A*	*C**AC****	***A*****T	*****T	400
FRV1	351	*****G**G	*****A*	***AC**G**	C**A*****T	*****T	400
VA	351	***C*T***	*****	***A**G**	***A**T**A	*****C*AC*	400
AU- 1	351	*****G	*****A*	***AC**G**	C**A*****T	*****	400
KUN	351	**GC*T**	*****G**A*	***A**G**	***A**C**A	T*C***AAT	400
GRV	401	TAATAATIAA	ACCAGTTGAT	AAAATIGATA	TGTCACAGGA	ATTCATCAA	450
NCDV	401	*G**G**ACG	*G****A**C	G****A****	**A*GA****	*A*T**C***	450
RRV	401	*G*****	*****C	*****	*****A**	**T****G	450
FRV1	401	*G*****	*****C	*****	*****A**	*****G	450
VA	401	*G***C**G	*****C	GTT**A****	***GA****	*****G	450
AU- 1	401	*G*****	*****	*****	*****A**	*****G	450
KUN	401	*G**G*GCG	*T*G*C**GC	GAG**A****	**A**A**A**	*A*T*****	450
GRV	451	AAACACTTIA	AAACGCTGAA	TGAATGGGCT	GAAGTIGAAA	ATCCATATGA	500
NCDV	451	**GA**G*G*	G*****AG*	A*****AA	A*T**AA***	***T****	500
RRV	451	***T*T**C*	*****A**	**T*****	*****	*****	500
FRV1	451	*G***A****	*****A**	***G*****	*****	***G****	500
VA	451	**A**A*C*	*****A*T	*****AG	AGT**AA***	*****	500
AU- 1	451	*G***A****	*****A**	***G*****	*****	***G****	500
KUN	451	**GA**C*A*	G*****AG*	A**G****AG	AGT**AA***	***T****	500
GRV	501	GCCAAAGGAA	GIGACIGCAT	CATIG			550
NCDV	501	A*****A***	*****G	*GA**			550
RRV	501	A*****A**G	*****	****			550
FRV1	501	A*****A**G	*****	****			550
VA	501	A**GTCA***	*****	*C***			550
AU- 1	501	A*****A**G	*****	****			550
KUN	501	A*****A***	*****G	**A**			550

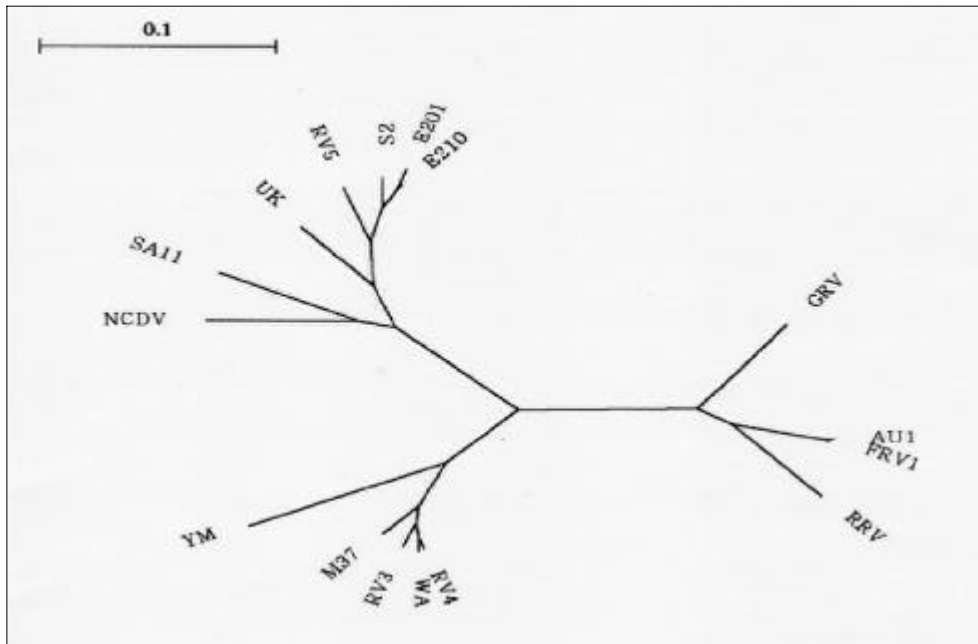
2- 4- 5. Comparison of NSP4 nucleotide sequence between GRV and other strains

GRV	1	NEKLTDLNYT	LSVITLMDNT	LHTIMEDPGM	AYFPYIASVL	TVLFILHKAS	50
NCDV	1	*****	S*****S*	****I****	*****	*****	50
RRV	1	*****	**V*****	*****	*****	*****	50
FRV1	1	*****	*****	*****	*****	*****	50
VA	1	*D**A****	****S****	**S*IQ****	***I*****	*****	50
AU- 1	1	*****	*****	*****	*****	*****	50
KUN	1	**F*****	*****S*	***I****	*****	*****	50
GRV	51	IPITKIALKT	SKCSYKVIKY	CIVSIFNILL	KLGYKEQIT	TKDEIEKQMD	100
NCDV	51	*****	*****V**	***T*****	*****	*****	100
RRV	51	V*****	*****	*****	*****	*****R**	100
FRV1	51	*****R*	*****	*****	*****	*****	100
VA	51	*****	*****	***T*I****	*****V*	*****Q**	100
AU- 1	51	*****R*	*R*****	*****	*****	*****	100
KUN	51	*****	*****V**	***T*I****	*****	*****	100
GRV	101	RVVKEMRRQL	EMDKLTIRE	IEQVELKRE	HDMLIKPVK	KIDMSQEFNQ	150
NCDV	101	*****	*****	*****I	**K*N*RA*D	E***TK*I**	150
RRV	101	*****	*****	*****I	*****D	*****	150
FRV1	101	*****	*****	*****I	*****D	*****	150
VA	101	*I*****	*****	*****I	**N**IR**D	V***K****	150
AU- 1	101	*****	*****	*****I	*****D	*****	150
KUN	101	*****	*****	*****I	Y*K**VRSTS	E***TK*I**	150
GRV	151	KHFKITLNEVA	EGENPYEPKE	VIASL			200
NCDV	151	*NVR**E**E	N*K*****	***AM			200
RRV	151	*Y*****D**	*****	*****			200
FRV1	151	RQ*****	*****	*****			200
VA	151	*NI***D**E	S*K****S*	***M			200
AU- 1	151	RQ*****	*****	*****			200
KUN	151	*NVR**E**E	S*K*****	***AM			200

2-4-6. Comparison of NSP4 amino acid sequence between GRV and other strains

6. GRV NSP4 Phylogram

sequence NCDV, UK, E210, E201, S2 FRV1 TRICON PROGRAM (2-4-7). NSP4 type YM, M37, RV3, WA, RV4가 FRV1, AU-1, RRV가 type nucleotide Wa, AU-1, phylogram



2-4-7. Phylogram of rotavirus NSP4 genes

7. NSP4 VP4 binding domain sequence  
 NSP4 phylogram 가 conserved  
 NSP4 sequence VP4 binding domain 131-148  
 RRV, FRV1, AU-1, M37, Wa, NCDV, SA11, UK NSP4  
 type AU-1, FRV1 RRV  
 18 M37 Wa 13 가 , NCDV  
 UK 10 가 SA11 6  
 GRV RRV, RRV1, AU-1 group  
 ( 2-4-8).

	131																		148
GRV	G3P3	H	D	M	L	I	I	K	P	V	D	K	I	D	M	S	Q	E	F
RRV	G3P3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
FRV1	G3P9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
AU-1	G3P9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M37	G1P6	-	-	N	-	-	T	R	-	-	-	V	-	-	-	-	-	K	-
Wa	G1P8	-	-	N	-	-	T	R	-	-	-	V	-	-	-	-	-	K	-
NCDV	G6P1	-	-	K	-	M	-	R	A	-	-	E	-	-	-	-	T	K	-
SA11	G3P1	Y	-	K	-	T	V	Q	T	T	G	E	-	-	-	-	T	K	-
UK	G6P5	-	-	K	-	M	-	R	T	-	-	E	-	-	-	-	T	K	-

2-4-8. Comparison of variable region of VP4 binding domain of the GRV NSP4 gene with those of other rotaviruses

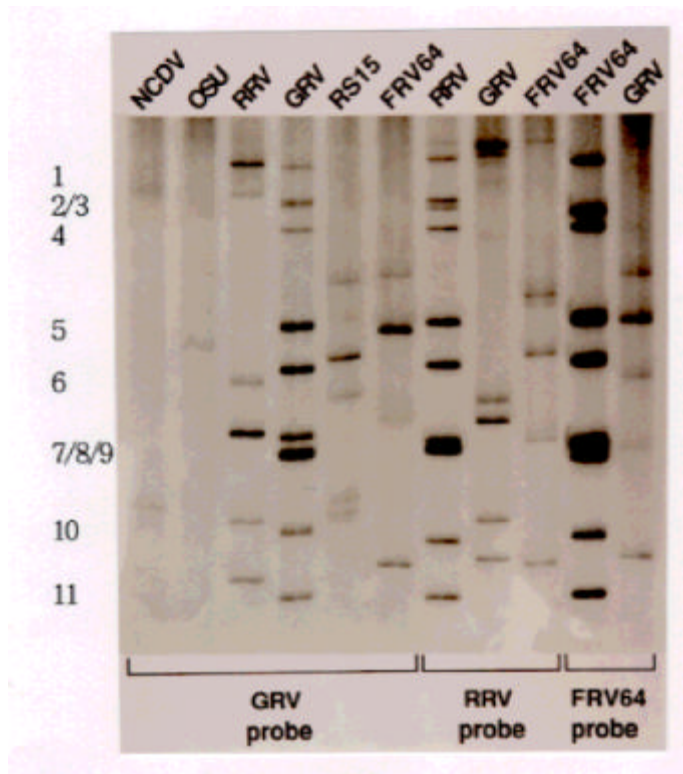


8. RNA-RNA hybridization genotyping

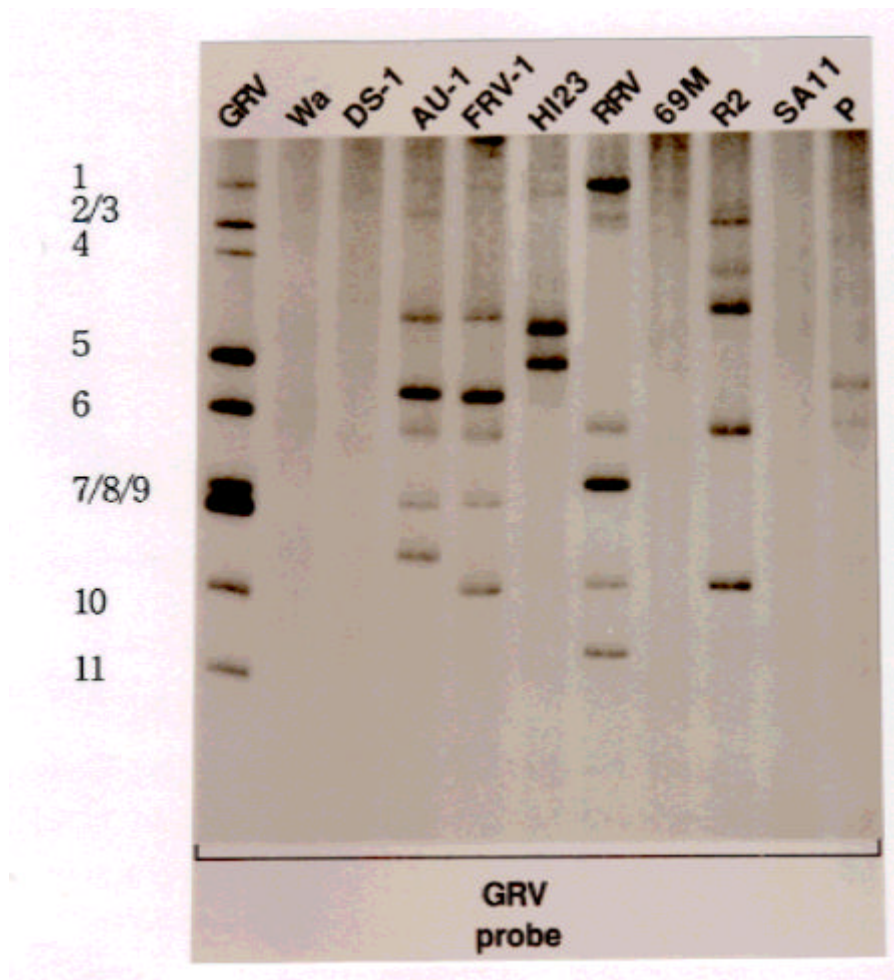
GRV, FRV, RRV  $^{32}$ P RNA probe, Wa, AU-1,  
 FRV-1, FRV64, RRV, R2, NCDV, OSU, Eb dsRNA ssRNA  
 hybridization 4% stacking gel 10% polyacrylamide gel  
 x-oma AR films

GRV probe Wa, Ds-1, 69M, R2, P, SA11,  
 RRV, FRV-1, NCDV, OSU hybridization Wa,  
 Ds-1, 69M, SA11, NCDV, OSU hybrid가, RRV 6 7  
 , AU-1 6, FRV-1, R2, RS15 5, R2, FRV64 4 hybrid가

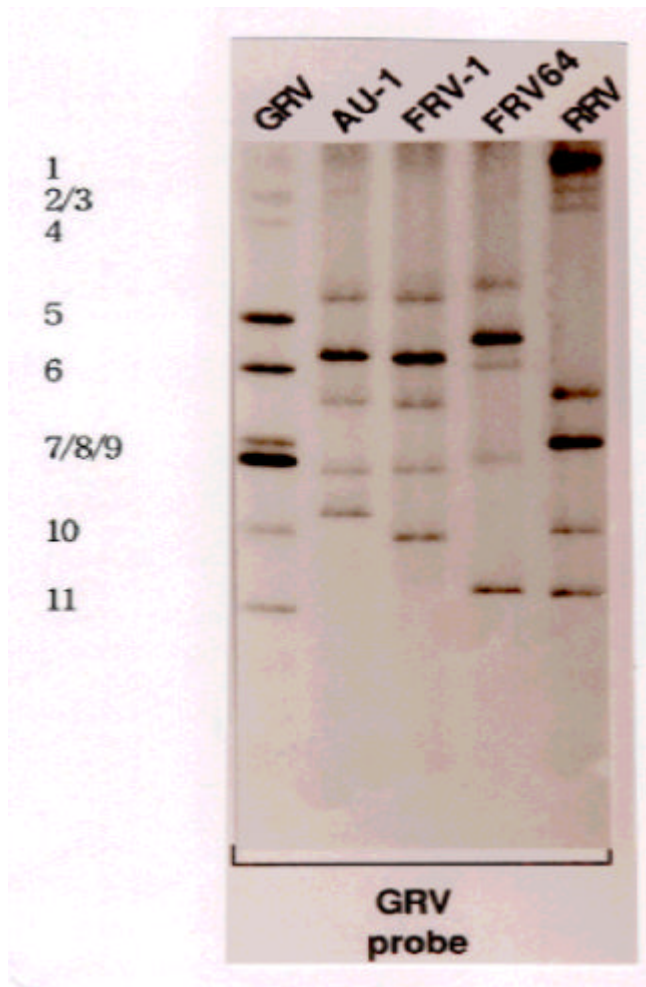
RRV probe RRV, FRV64 panel hybridization 5 6 hybrid  
 , FRV64 GRV hybridization 5 hybrid가  
 ( 2-4-9; 2-4-10). GRV  
 AU-1, FRV-1, FRV64, RRV ( 2-4-11).



2-4-9. RNA-RNA hybridization of GRV  
with animal rotaviruses



2- 4- 10. RNA- RNA hybridization of GRV with other rotaviruses.



2- 4- 11. RNA-RNA hybridization of GRV with relative rotaviruses



(Merchant 1991, Offit Clark 1985, Shaw 1993),

105 106

가 (Greenberg 1986).

cytotoxic T lymphocyte (CTLs)가

, memory TLs

(Offit 1991). memory TLs IL-2

IL-4, IL-5 . -specific helper T cell

lamina propria Peyer's patch

(Offit 1992).

VP4 VP7 (Offit

Blavat 1986, Offit 1986, Hoshino 1985).

baculovirus, herpes simplex virus, adenovirus, vaccinia virus vector

VP4 VP7

가 (Andrew 1990, Dormitzer 1992,

Nishikawa 1989). VP4 VP7 epitope

(Matsui 1989).

CD8+ cytotoxic T lymphocytes (CTLs)가

(Offit Dudzik 1990, Dharakul 1991).

CTLs VP4 VP6 가 VP7 G types

(Offit 1994, Offit Dudzik 1988). CD8+ T cells

(Franco 1997).

immunoglobulin A (sIgA)

( sIgA)가 가

(Kapikian Chanock 1990, Offit 1994).

(Wyatt 1979).

(Santosham 1991, Hoshino 1994),

(Midthun 1985, Christy 1993, Glass

1994)가 . 가

(Ward 1986, Clark 1986, Anderson 1986, Vesikari 1985, Flores 1988, Midthun 1985, Vesikari 1983).

(Green

1990, De Mol 1986, Hanlon 1987).

(NIH)

(RRV)

25%

14- 31% seroconversion 50%

(Bernstein 1995, Rennels 1996).

1988 8 31 FDA "Rotashield"

VP4 VP7 reassortant (Vesikari 1997, Kapikian 1996) 2, 4, 6  
2 .

1999 7 15

(Robert 1999).

가

가

가

(Ceyhan 1993, Friedman 1993).

VP4 VP7

(McCrae 1987, McGonigal 1992,

Offit 1994). (Baculovirus) RRV VP4  
 .  
 (Mackow 1989, 1990).  
 VP7 가 VP7  
 .  
 가 (Andrew 1990,  
 1992, Both 1993).  
 Crawford (1994) VP2 VP6 VP6 VP7  
 가  
 ( ,  
 , ) VP4  
 VP7 가 ,  
 가 (Fernandez 1995, Saif  
 1995).  
 TF104 ,



1.

2

TF104

100

10

agarose

plaque picking

plaque picking

2

2.

100

106 TCID<sub>50</sub>

100

1

104 TCID<sub>50</sub>/M<sub>0</sub>

6

3.

2

culturette

1M<sub>0</sub>

PBS

1200rpm

10

0.2μ<sub>l</sub>

filter

TF104

CO<sub>2</sub> incubator

2 3

CPE가

4. ELISA

가

ELISA

Anti- goat immunoglobulin

Ammonium sulfate

가

가

30

가

3000rpm

30

PBS

ammonium sulfate .  
 complete Freund's adjuvant . 3 가  
 가 , SDS- PAGE  
 heavy chain incomplete Freund's adjuvant  
 . 2 가  
 Horse reddish peroxidase Fisher 가  
 coupling . ,  
 ELISA anti- goat globulin .  
 PBS 1Mℓ , coating plate well  
 100μℓ . 1 PBS 3  
 (rabbit anti- goat  
 immunoglobulins) 1 . Substrate 가  
 ELISA reader .  
 5.  
 7 (106  
 TCID<sub>50</sub>/ml) . 3

1.

TF104 77  
 3-1 가  
 3 가 2 가 66%  
 , 77  
 가 100 가  
 36%  
 106 T CID50 1M $\emptyset$

3-1.

	( )	(%)	(%)
1	5	3(60)	2(66)
77	5	3(60)	0
100	19	7(36)	0
	5	0(0)	0

2.

19  
 1ml 106 T CID50 2  
 , 3-2 19 14 12  
 , 6

가 ml  
 100 1 104 TCID50가  
 2 2 2 14  
 가 100 5 95  
 , 95 가 14

3-2.

Goat (Tag No)	Age (month)	Diarrhea post vaccination (days)							
		0	2	4	6	8	10	12	14
52	3								
53	3								
54	3								
56	3								
57	>12								
60	3								
61	3								
68	3								
79	>12								
80	3								
86	3								
87	3								
88	>12								
89	3								
90	>12								
91	12								
92	>12								
96	>12								
98	>12								

3.

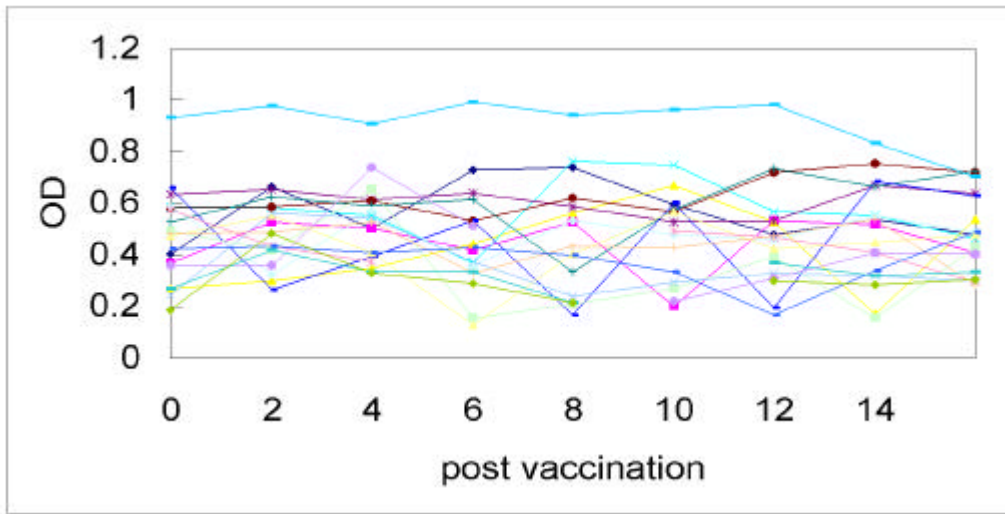
100

20

( 3-1).

, 가 .

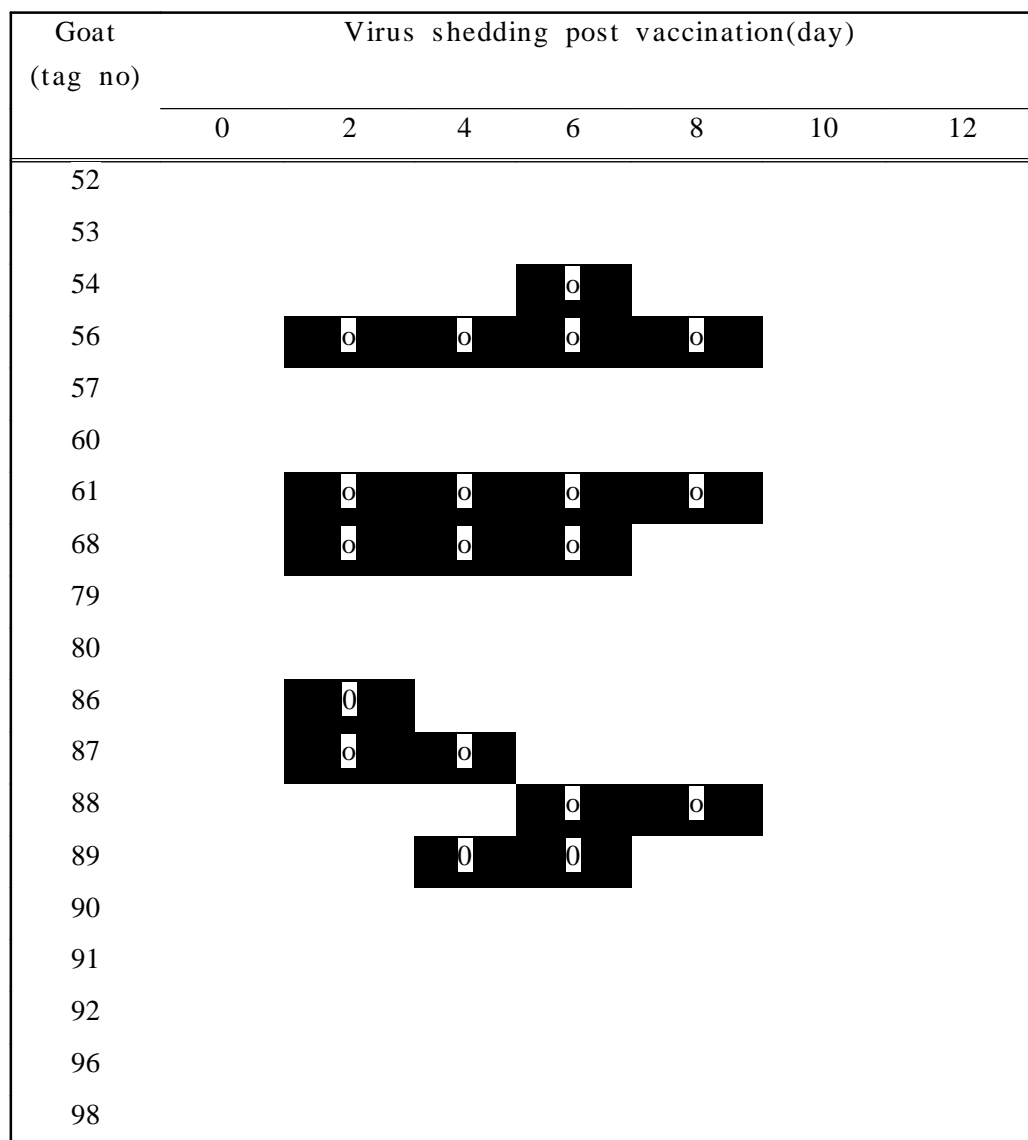
3-1.



4.

, 3-2  
19 8 가  
2 6  
2 8  
8

3-2.



5.

100  
106 TCID<sub>50</sub>/ml

7

15

3-3

가

가

4 3

3-3.

Goat(No. tag)	Diarrhea	Death	Discoloration of skin hair
54	-	-	-
56	-	-	-
61	-	-	-
68	-	-	-
86	-	-	-
87	-	-	-
88	-	-	+
control	+	+	+
control	+	-	+
control	+	-	+
control	-	-	-

4

, 가

가 가 가 가

*Pasteurella hemolytica*

, *Toxoplasma*

40% 가 20% 가 20% 가

670,000 (

. '97. ) 1/5 5

3 가 가 가 가

25% ( . '96.

),

가 가

가 1)

가



2)

3) ,

4) 5)

6) .

1997 11 , 200

164 .

13 , 3 가 .

가 가 7 50 가

, 4 25 가

. 2 13 가 .

가 95 .

가

가

가

, 7

, 가 가

, 1 .

plate tube agglutination test .

가 가

가 가

.  
*Shewanella putrefaciens*, *Burkholderia cepatica*, *Kinella denitrifican*, CDC-group II, *Actinobacter radioresistens*, *Kingella*

kingae .

44% ,

26% ,

8%

1 ,

가 ,

1-4, ,

1-5

Bovine

viral diarrhea virus

Bovine respiratory syncytial virus

2

가

가

가

가

가

가

10 100

(FAO, '94. 4), 가

20%

가

가

(Proceeding of a workshop held at the

university of Queensland, Brisvane, Australia. '84).

*Cryptosporidium* *E. coli, Salmonella, Clostridium,*  
*coronavirus* *rotavirus,*  
(Munoz, 1996),  
6.1% 30  
60% 가 (Vet. Rec. '97).  
6.1%  
30 60%  
가 (Munoz 1996). West  
Indies Trinidad 28.6% 가  
(Kaminjolo 1994).  
atypical rotavirus 가 (Couacy  
1995).  
Reoviridae . 15  
common inner capsid 가  
80 ELISA immunofluorescence  
가 . common group 가  
typical rotavirus 가 atypical rotavirus  
major inner capsid protein RNA PAGE A G  
7 serogroup . A group typical rotavirus B G

atypical rotavirus B C가 .  
 가 group A  
 B가 atypical rotavirus 가 .  
 1943 (Light Hodes 1943). 1969  
 Reovirus 가 (Mebus 1969),  
 (Flewett others 1973), (Rodger 1975), (Els Lecatsas 1972),  
 (Muce Zajac 1972), (Tzipori 1976) (Flewett 1975)  
 가 .  
 1973 가 (Bishop  
 1973), 1963 가  
 (Adams Kraft 1963),  
 (SA11)가 ,  
 SA11 가 (Malherbe  
 Strickland- Cholmley 1967). Reovirus Reovirus  
 가 (Flewett 1974). 1976  
 (Woode 1976)  
 1976 Scott 가  
 (Snodgrass 1976).  
 1971 (NCDV)  
 (Mebus 1971), 가  
 (Albrey Murphy 1976, Banatvala 1975, Welch Twiehaus 1973,  
 Wyatt 1974). 1977 trypsin  
 (Babiuk  
 1977).

TF104 CPE가 , TF104 TF104 가 VP4

가 79 100%

가 가 2-2-1

가

가 2 가 2 , 2

1

2-3-1). (

TF104 77 5 3

VP7, VP4, NSP4 RT-PCR

DNASIS program ,

VP4 2,328 nucleotide 776

NCDV, SA11

가 FRV1 3

가 1 가 .

VP4 genotype P type

, VP4 20

P type RRV 90%

P type P3 가 .

가 . , P serotype P5가 가

P11 가 (Suzuki 1993, Chang 1996)

G P type G6P5, G6P11, G10P1

. G6P5 type 가

, A, B C

( 1998), G5 P7

interspecies transmission natural reassortments가

(Kang 1997). P11

( 1998).

VP7 NCDV, SA11, AU-1 978 nucleotide

326 , VP7 type (G type)

14 type HCR3, SA11

95% type 3 가 가 . VP7 14 type

HCR3

SA11 가 , RRV

G3 type subgroup

. 14 G serotype 가 (Estes 1996),

Great Britain (Snodgrass 1990), Germany

(Brussow 1994), Argentina (Bellinzoni 1989), the United States (Parwani

1993), Thailand (Taniguchi 1991), Australia (Huang 1992) 가

가 G6 G10 G1  
 (Blackhall 1992) G8 (Snodgrass 1990, Taniguchi 1993)  
 G2, G3, G11

NSP4  
 NCDV, RRV, FRV-1, Wa, AU-1, KUM 525 175

NSP4 phylogram  
 group group  
 AU-1, FRV1, RRV가 group 가 가

NSP4 가 VP4 binding domain 112-146  
 group RRV, RFV1, AU-1, M37, Wa, NCDV, SA11, UK  
 RRV, FRV-1, AU-1 100%가 NSP4 Type

P type, G type  
 RNA-RNA hybridization genotype  
 RNA polymerase in vitro virus  
 RNA mRNA probe  
 genogroup

Wa, KUN, AU-1 3 genogroup

GRV, RRV, FRV64 probe , , ,  
 hybridization AU-1, FRV-1, FRV64,  
 RRV 가 hybrid band가 .  
 가

가 type ,  
 가 가 가

transmission 가

G6 G8

(Gouvea 1944), G6

(Gerna 1992), G8 type

(Parwani 1993). G10

interspecies transmission 가

(Pongsuwanna 1996), Parwani (1992) Suzuki (1993)

G/P strain

G P type 가 natural reassortment

bovine rotavirus

(Urasawa 1990, Gerna 1992)

VP7 G3 SA11

가 가 ( 1998). G3 SA11

VP7

RRV

, VP4 VP7, NSP4

가 type 가

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