

Epidemiology and Molecular Biological Characterization of Rabies in Korea

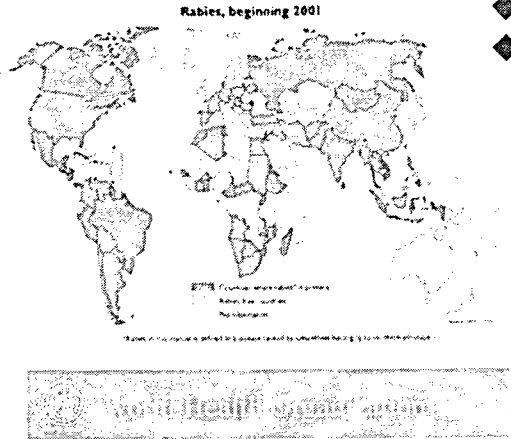
Bang-Hun Hyun

(Virology Division, National Veterinary Research and Quarantine Service)

Rabies is caused by highly neurotropic viruses, most belonging to a single serotype of the genus *Lyssavirus*, family *Rhabdoviridae*. In South Korea, urban rabies cases were first reported in 1907 and had an annual average of 500 endemic cases between 1907 and 1945. Between 1950 and 1984, rabies outbreaks decreased significantly to an annual average of 30 cases and were eradicated in 1985 due to the vaccination campaign for dogs. In 1993, unfortunately, recurrence of sylvatic rabies was reported in Gangwon province, which is the northern border area of South Korea. During the years of 1994-2005, rabies endemic areas expanded 18 counties, and 389 animal and 6 human rabies cases were registered in Gangwon and Gyeonggi provinces near the demilitarized zone. Raccoon dogs (*Nyctereutes procyonoides koreensis*) is the main epidemic carrier of rabies in Korea.

A molecular epidemiological study was performed on thirteen Korean virus isolates which were collected from wild and domestic animals diagnosed as rabid between 1998 and 2004. The study was carried out based on the comparison of nucleotide and amino acid sequences of nucleoprotein (N) and glycoprotein (G) coding regions and nucleotide sequence of the G-L intergenic (Ψ) non-coding region of the isolates. Phylogenetic analyses of the isolate showed that they formed a monophyletic group closely related to the Arctic strains but distant from other Asian strains, including Chinese strains. The Korean isolates can be divided into two subgroups. All the topology of the most likelihood tree of Korean isolates using nucleotide and amino acid sequences of N, G and G-L region reflected not the species but the year of isolation and geographical location of the virus isolates.

Distribution of Rabies

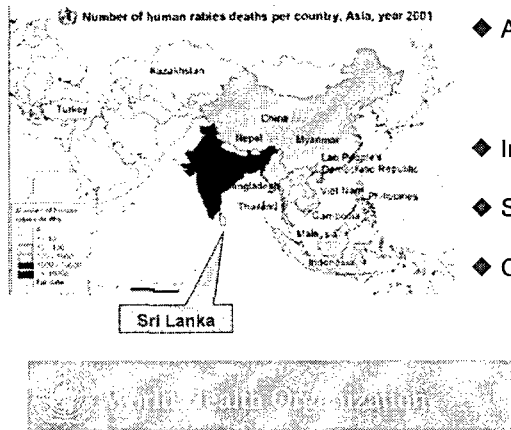


◆ World wide outbreaks

◆ Free countries

- Australia
- New Zealand
- Japan
- Hawaii / Guam
- United Kingdom
- Ireland
- Norway
- Sweden
- Belgium
- Italy
- Portugal

Rabies in Asia



◆ Annual number of deaths

- 55,000
- Mostly in rural areas of Africa and Asia

◆ In Asia

- 31,000 (56.4%)

◆ Spent US\$ 560 million each year

- On post-exposure prophylaxis

◆ Canine rabies is endemic

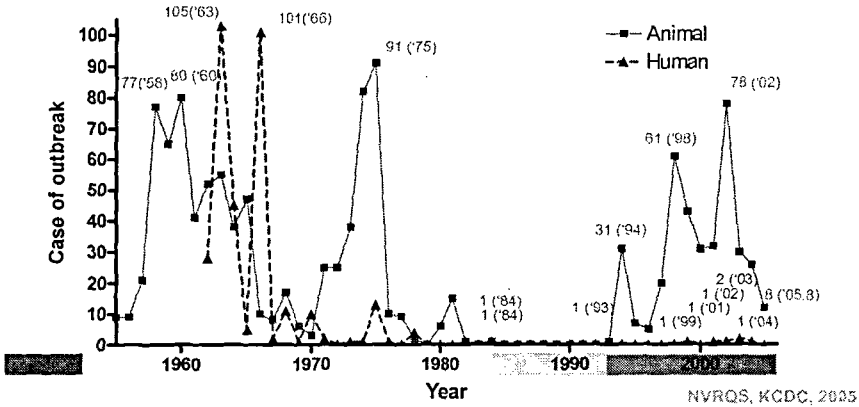
- India and Sri Lanka

2001, WHO

1. Rabies in Korea

Rabies outbreaks in Korea ('55-'05.8)

- 1907 : First record of outbreak
- 1907 ~ 1945 : Endemic stage (100-900 animal case, annually)
- 1945 ~ 1992 : Decrease and elimination stage
- 1985 ~ 1992 : No outbreak
- Since 1993 : Recurrence stage (1995, Hwang)

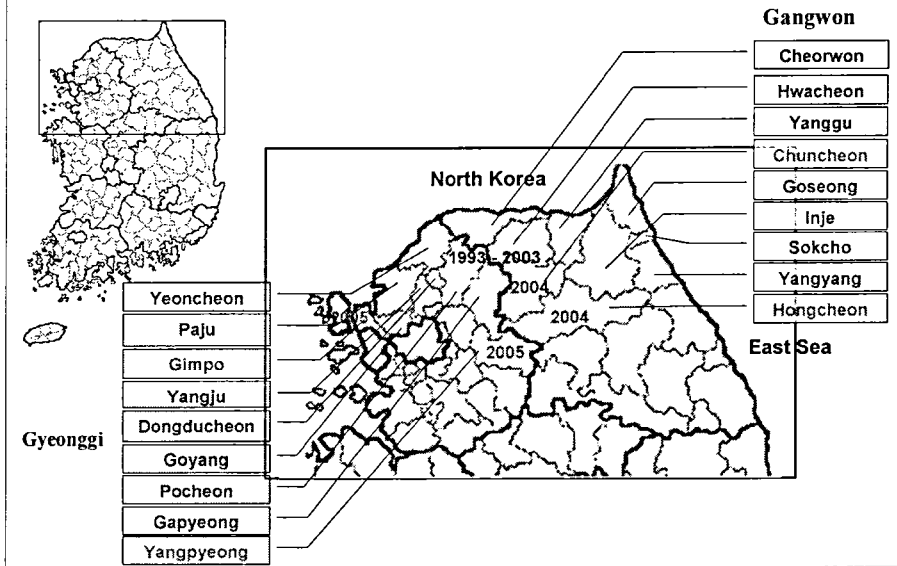


Human rabies cases since 1999 in Korea

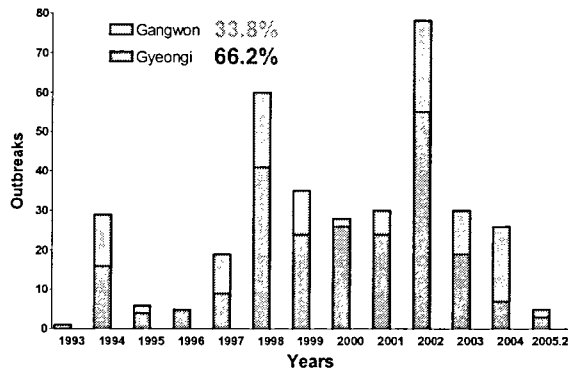
Year, month	Case	region	Exposure / incubation period	Post exposure Immunoprophylaxis
1999	M/53	Paju, Gangwon	Bite by suspected rabid dog?	no
2001.12	M/68	Hwacheon, Gyeonggi	arm bites by suspected rabid raccoon dog / 11wks	no
2002.6	M/46	Yeoncheon, Gyeonggi	face bites by suspected rabid dog which struggled with raccoon dog / 5wks	no, But immunoglobulin was administered for symptom delay
2003.2	M/60	Pocheon, Gyeonggi	face bites by suspected rabid dog / 3wks	yes, But immunoglobulin was improperly administered
2003.5	M/44	Pocheon, Gyeonggi	face bites by suspected rabid raccoon dog / 8wks	no
2004.5	M/72	Koyang, Gyeonggi	arm bites by suspected rabid dog / 11wks	No, (probable case)

KCDC (internal document) 2005

Region of Rabies outbreaks

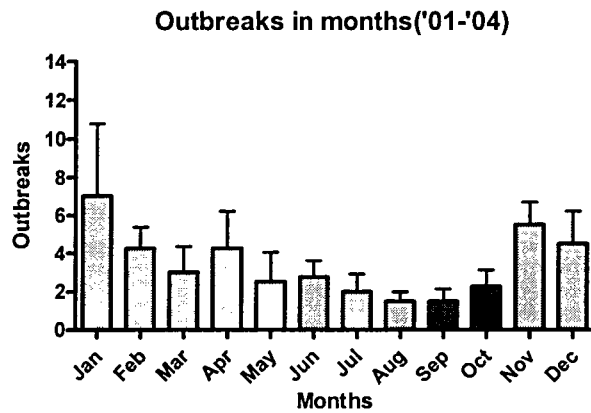


Rabies outbreaks in Korea (animal)

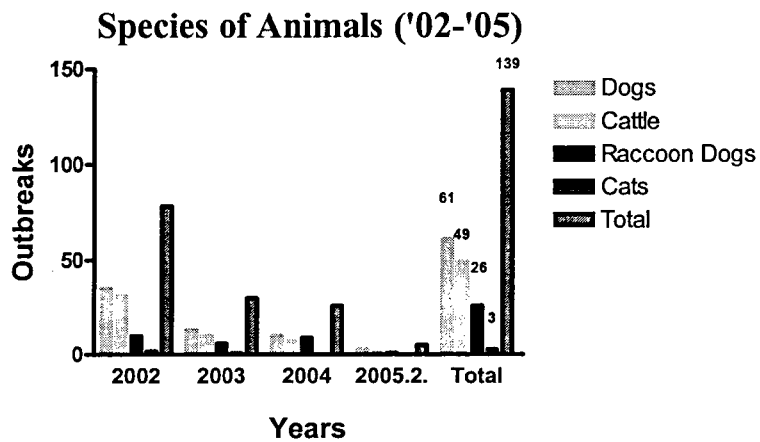


Regions	Years													Total
	'93	'94	'95	'96	'97	'98	'99	'00	'01	'02	'03	'04	'05	
Gyeonggi	0	16	4	5	9	41	24	26	24	55	19	7	3	233
Gangwon	1	13	2	0	10	19	11	2	6	23	11	20	2	119
Total	1	29	6	5	19	60	35	28	30	78	30	27	5	352

Rabies outbreaks in Korea (animal)



Rabies outbreaks in Korea (animal)



Collection of Rabies specimen

- Species and regions
 - ✓ Positive samples : Total 19 (1998-2004)
 - ✓ Species : Raccoon dogs 8, Dogs 5, Cattle 6
 - ✓ Regions : Gyeonggi-do and Gangwon-do, 9 province
 - ⇒ Paju (2), Goyang (2), Yeoncheon (1)
 - Cheorwon (6), Hwacheon (3), Yanggu (1),
 - Chuncheon (4), Gosong (1), Inje (2)

Veterinary vaccine strain in Korea

Phenol-glycerine inactivated vaccine
(1922-1939 / 592,077 dogs)

- prepared from the brain and spinal cord of rabbits, and produced in Japan



Avianized attenuated live vaccine
(1960-1979)

- low egg passage (LEP) Flury strain

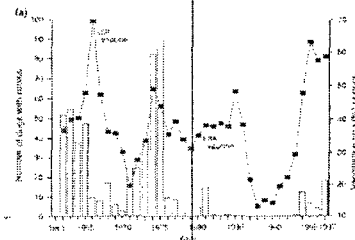


Tissue culture attenuated live vaccine
(1980-present)

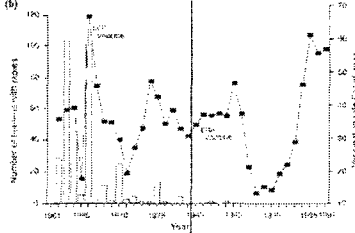
- Evelyne-Rokitnicki-Abelseth (ERA) strain

(1995, Hwang)

Dogs



Humans



(2001, Lee et al.)

Serological surveillance in husbandry animals

❖ Geographies

- Seoul and Districts (-Si or -Gun) where have broken out rabies
- Numbers of districts : 15 ('02) ⇒ 15 ('03) ⇒ 17 ('04) ⇒ 18 ('05. 1/2)
- '2005, 11. : 19 districts
 - Seoul, 18 districts in Gangwon-do (9) and Gyeonggi-do (9)

❖ Results

Years	Test numbers			Positive rate(%)	
	Dogs	Cattle	Total	Dogs	Cattle
1999	916	772	1,688	51.7	17.7
2002	491	213	704	43.1	25.1
2003	618	311	929	72.8	50.8
2004	703	348	1,051	78.0	56.9
2005.1/2	386	181	567	61.6	53.6

Label for vaccinated dogs (I)

(Test study)

❖ In 2004

- 4 districts : Yangju, Yeoncheon, Yanggu, Cunchoen
 - ⇒ Label : Individual (10,204 / 13,000 ea, 78.5%)
 - ⇒ Attachment board for recording
 - : Farm has more than 10 dogs (450 / 450 ea, 100%)

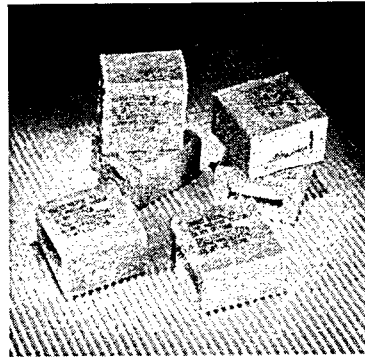
❖ In 2005

Areas		Label (rate)	Board
Seoul	Eunpyeong-gu	2,500	2
	Ganguk-gu	2,500	-
	Dobong-gu	2,500	-
	Nowon	2,500	-
Gyeonggi	Yangju-si	3,000	110
	Uijeongbu-si	3,150	40
	Goyang-si	1,100	10
	Guri-si	1,300	280
Gangwon	Taebak-si	1,550	80
	Hongcheon-gun	9,000	290
	Cheorwon-gun	5,550	300
Total		34,650	1,112

Bite Vaccine



- Vaccinia-rabies glycoprotein(V-RG)
 - Vaccinia virus : Copenhagen strain
- Rhone-Merieux
 - Raboral V-RG



Bait containing oral rabies vaccine
(dimensions 1 1/4" X 1 1/4" X 3/4")

Application of bait vaccines in Korea (1)

❖ Studies for bait vaccine (1999 – 2001)

⇒ Title : Rabies eradication plan using bait vaccine in Korea

- Infectious situation of Rabies in wild animal
- Ecological characteristics of wild animal
- Epidemiological survey in Gangwon and Gyeonggi province
- Field trial of Rabies bait vaccines / SAG-1 (Virbac) and V-RG (Merial)

❖ Results

- Only raccoon dogs showed positive reaction to rabies in FA test
- Safety and efficacy
 - Average antibody forming rate : 32% (10/31), 5 regions
- Population density : 2.9-8.1 individuals / km²
- Home range boundaries : 1.5 – 7.5 km²



Application of bait vaccines in Korea (2)

Years	Province	Regions	Quantity (Ea)	Ingestion rate (%)
2001	Gyeonggi	3	10,000	65
	Gangwon	2	10,000	75
	Total	5	20,000	70
2002	Gyeonggi	5	41,000	82
	Gangwon	2	36,000	60
	Total	7	77,000	72
2003	Gyeonggi	7	93,600	89
	Gangwon	7	112,800	81
	Total	14	206,058	85
2004	Gyeonggi	7	100,000	85
	Gangwon	9	120,000	87
	Total	16	220,000	86
2005	Gyeonggi	-	100,000	-
	Gangwon	-	120,000	-
	Total	-	220,000	-

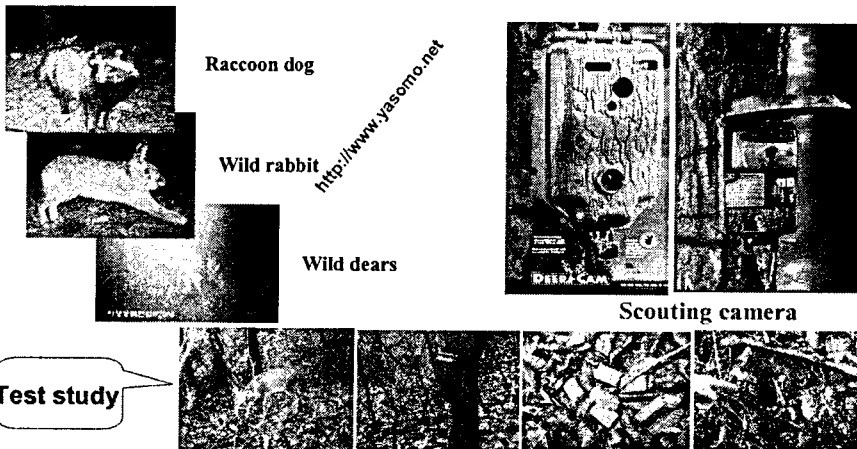
Wild animal monitoring for bait vaccine (III)

❖ Scouting camera methods

⇒ Period (Test study) : 2004. 11. – 2004.12.

⇒ Areas : Paju-si Jeoksung-myeon and Hongcheon-gun Dong-myeon

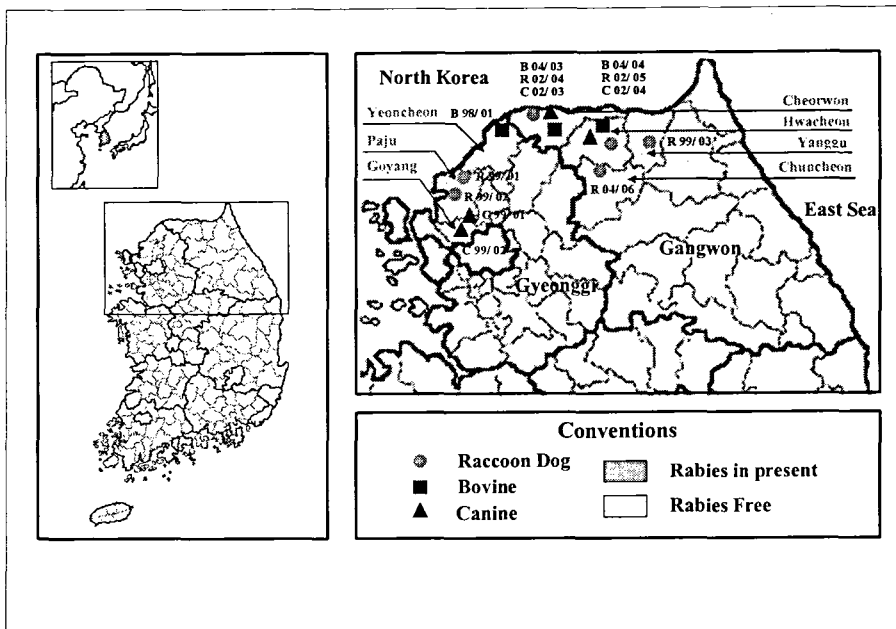
➤ DeerCam DC-200 (USA) / Maximum working distance :18m

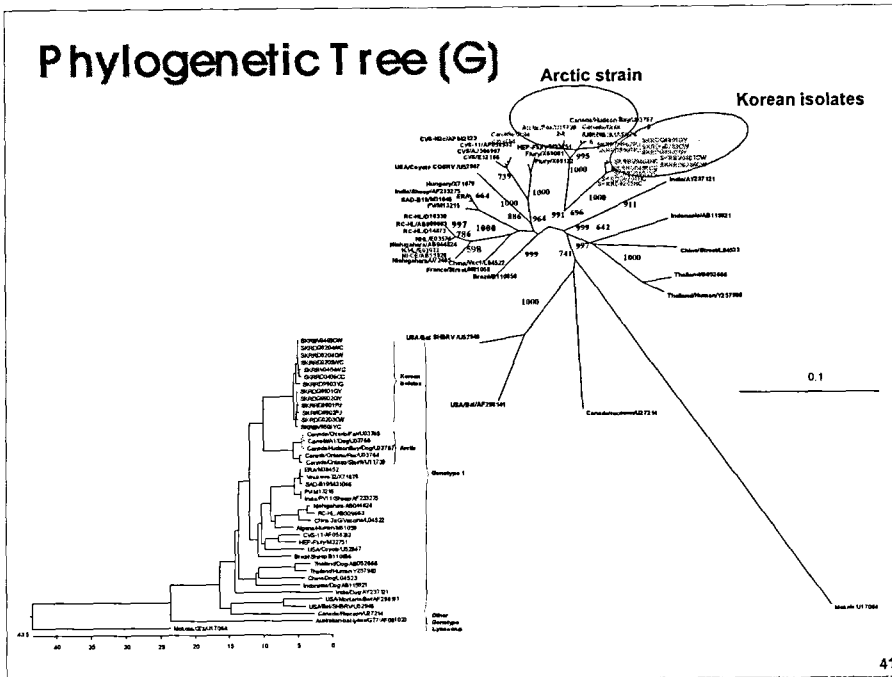
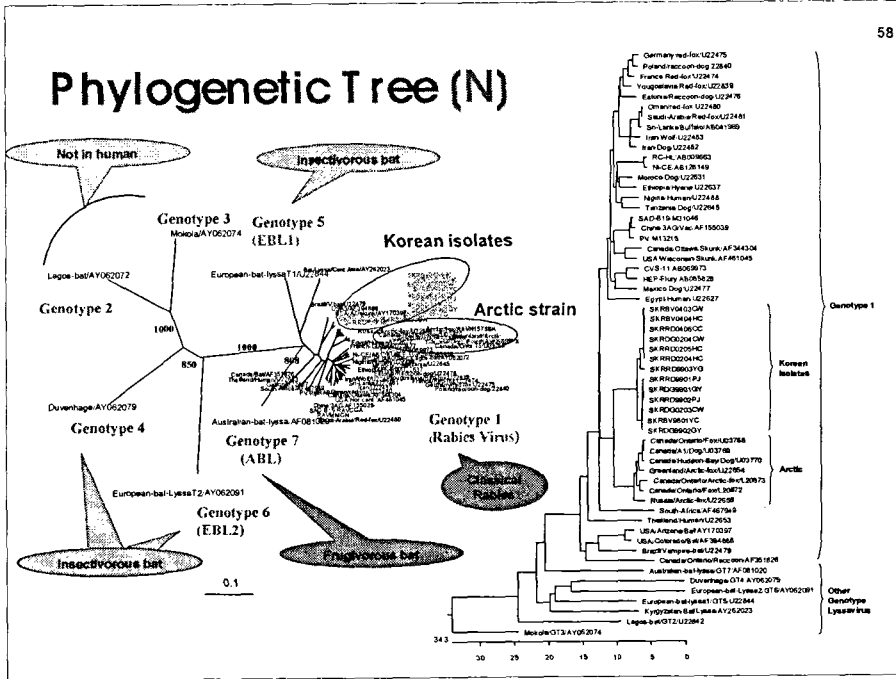


2. Molecular epidemiology of Rabies virus isolated from Korea

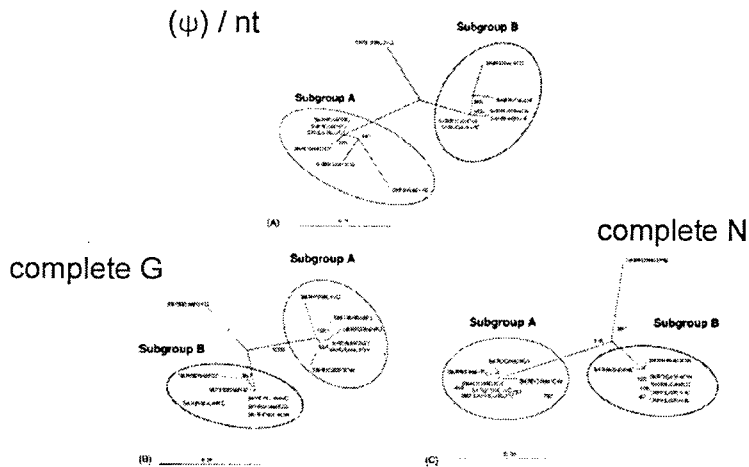
Rabies virus Isolated in Korea

Virus number	Virus code	Sample No	Species	Year	Locality
1	SKRBV9801YC	B98/01	Cattle	1998	Yeoncheon-gun
2	SKRRD9901PJ	R99/01	Raccoon Dog	1999	Paju-si
3	SKRRD9902PJ	R99/02	Raccoon Dog	1999	Paju-si
4	SKRRD9903YG	R99/03	Raccoon Dog	1999	Yanggu-gun
5	SKRDG9901GY	C99/01	Dog	1999	Goyang-si
6	SKRDG9902GY	C99/02	Dog	1999	Goyang-si
7	SKRRD0204CW	R02/04	Raccoon Dog	2002	Cheorwon-gun
8	SKRRD0205HC	R02/05	Raccoon Dog	2002	Hwacheon-gun
9	SKRDG0203CW	C02/03	Dog	2002	Cheorwon-gun
10	SKRDG0204HC	C02/04	Dog	2002	Hwacheon-gun
11	SKRRD0406CC	R04/06	Raccoon Dog	2004	Chuncheon-si
12	SKRBV0403CW	B04/03	Cattle	2004	Cheorwon-gun
13	SKRBV0404HC	B04/04	Cattle	2004	Hwacheon-gun

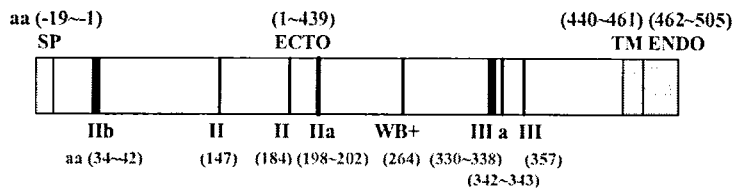




Radial Tree among the Korean isolate



Glycoprotein gene coding region



• Composition of glycoprotein

- SP (signal peptide) : Cleavage for mature glycoprotein
- ECTO (ectodomain) : B- & T-cell antigenic sites, receptor recognition, membrane fusion
- TM (transmembrane domain)
- ENDO (endodomain) : Interaction with internal viral, cellular protein

• Antigenic sites

- Major antigenic site (II, III), Minor antigenic site (a), Neutralizing linear epitope VI (WB+)
- II(aa184), III, WB+ sites : conserved
- IIb, II(147) : changed compared with PV or SAD-B19 or ERA strain
- All of the cystein residues : conserved
- The potential N-glycosylation site at position 37 and 319
- Arginine at position 333 : virulence



