

SFTS virus and other tick-borne pathogens in the Republic of Korea



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Contents

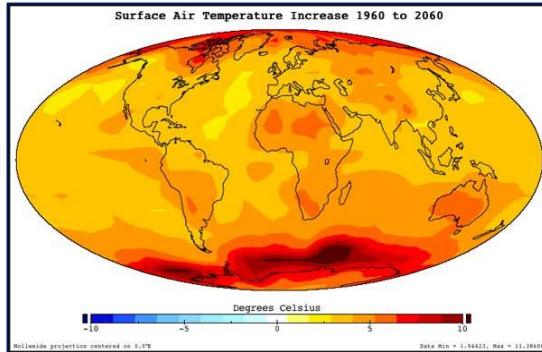
- Introduction
 - Climate change
 - Ticks in Korea
- SFTS virus
 - In ticks
 - In animals
- Other tick-borne pathogens
 - In ticks
 - In animals



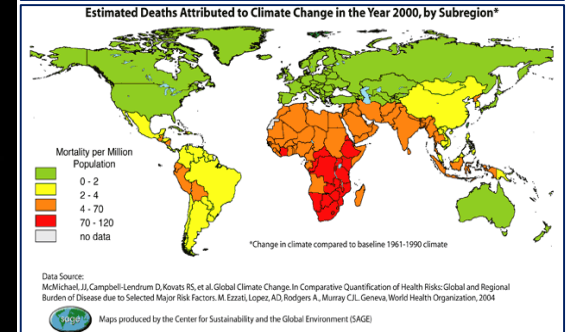
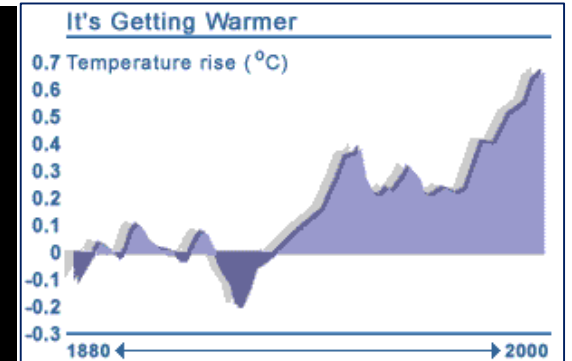
Global warming



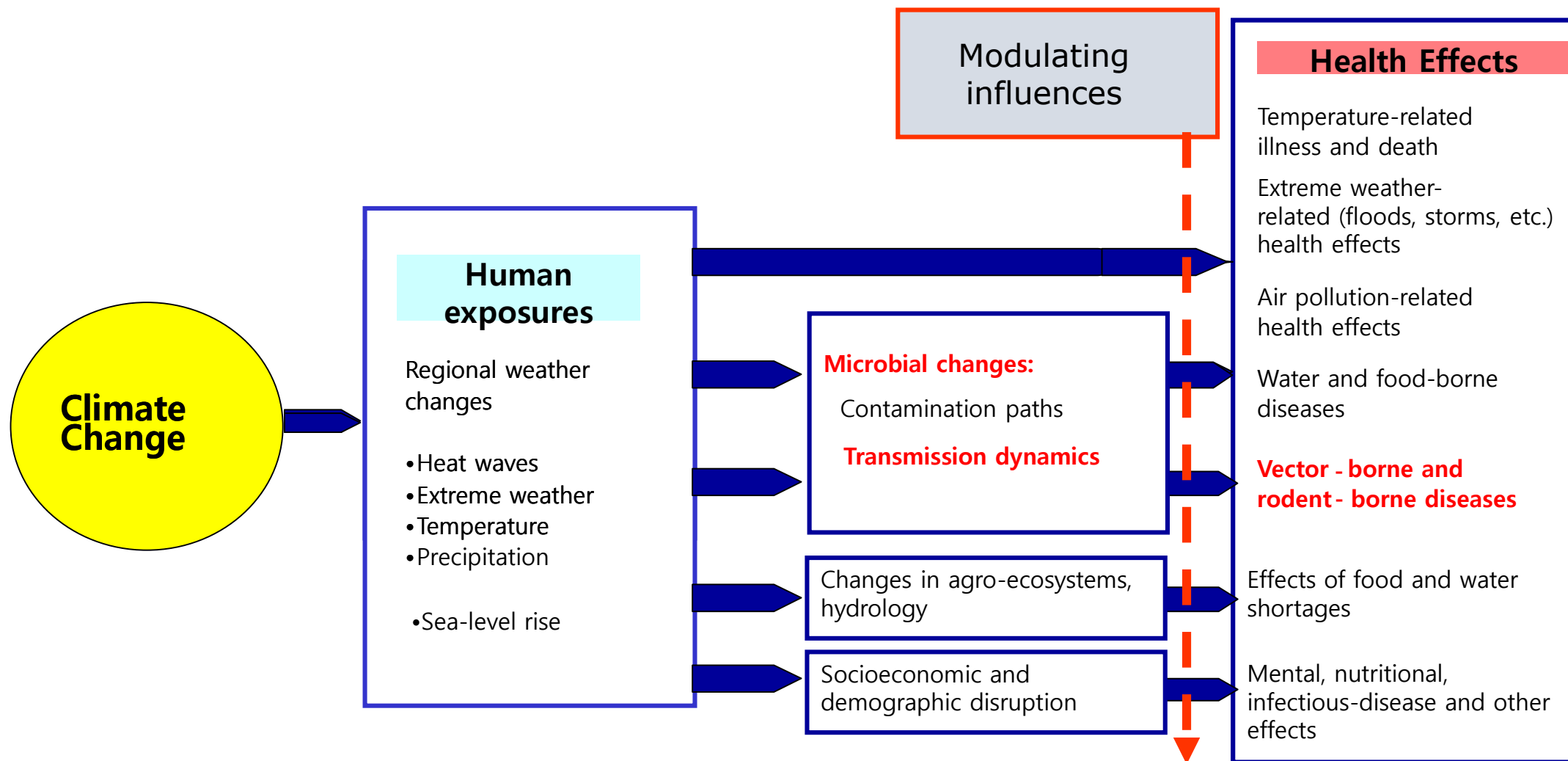
Climate change



www.whataretheywaitingfor.com/global-climate-change.html

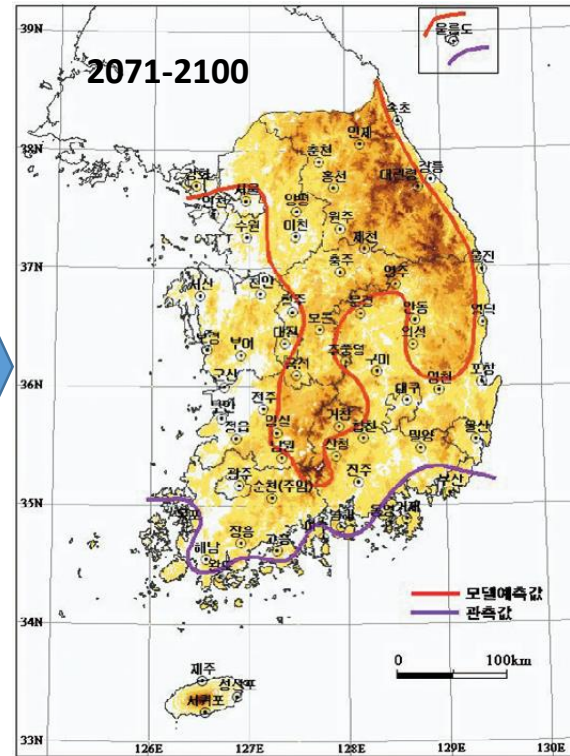
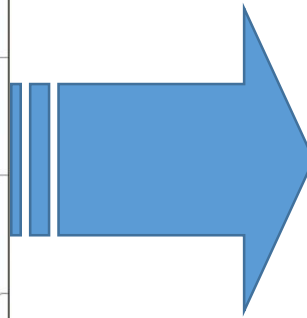
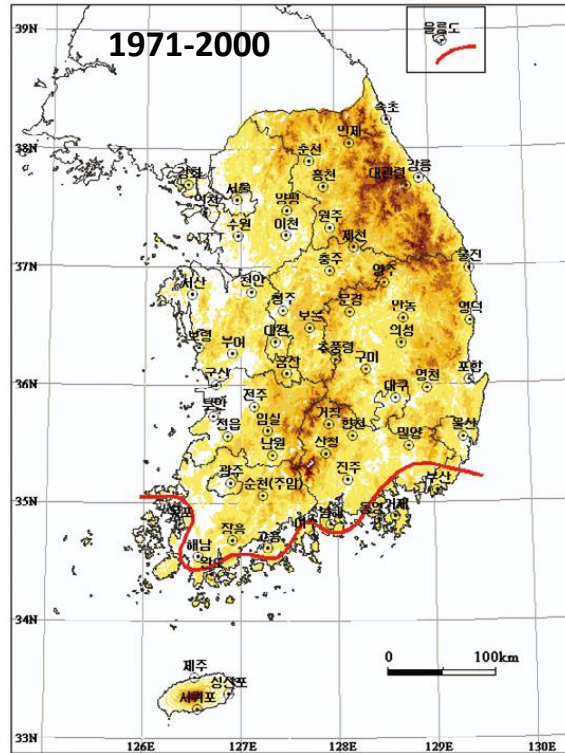


IPCC report about climate change and Health



Climate zone in Korea

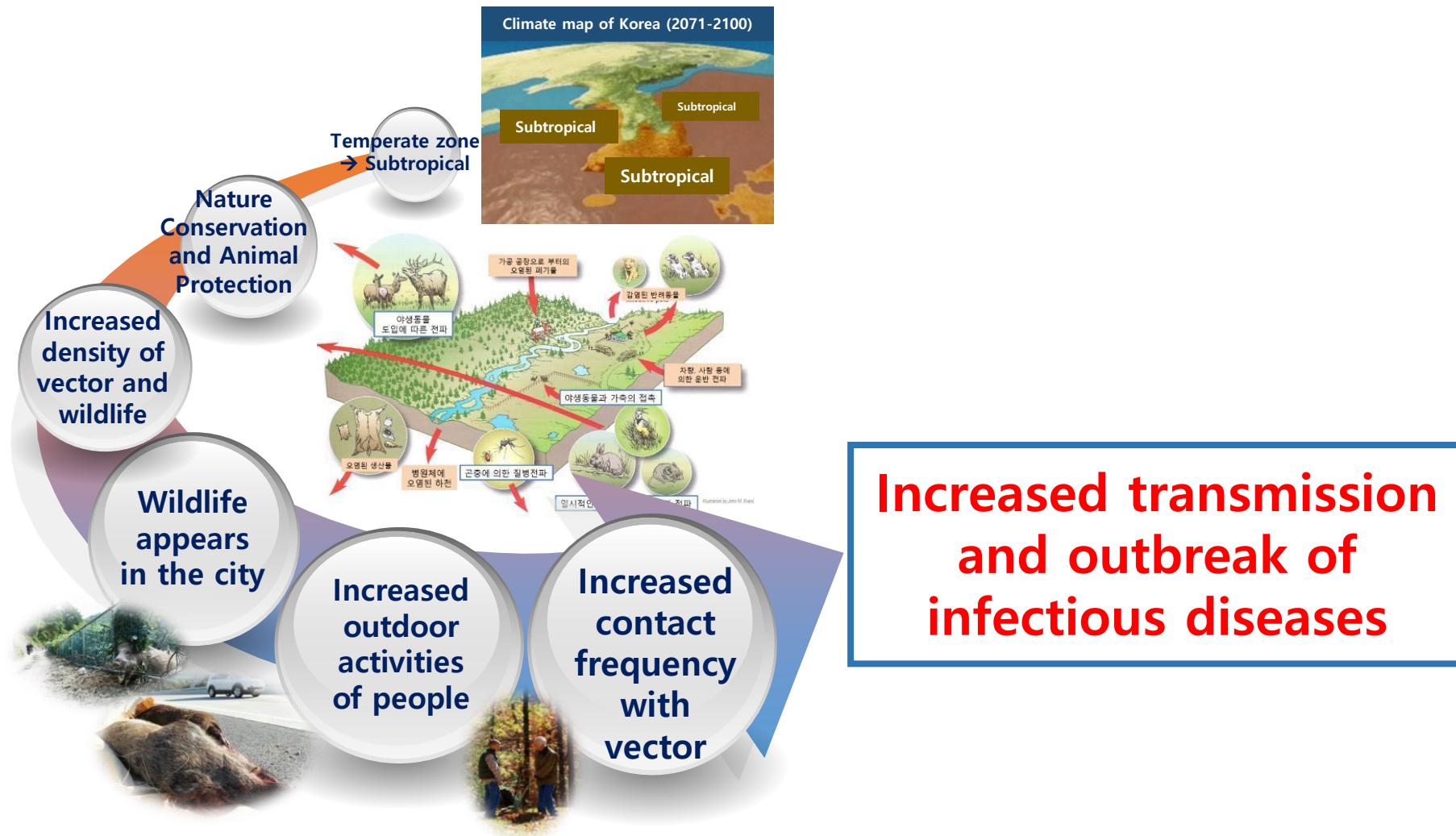
30 years(1971-2000)
Subtropical zone based on
observation mean values



(National Institute of Meteorological Research)

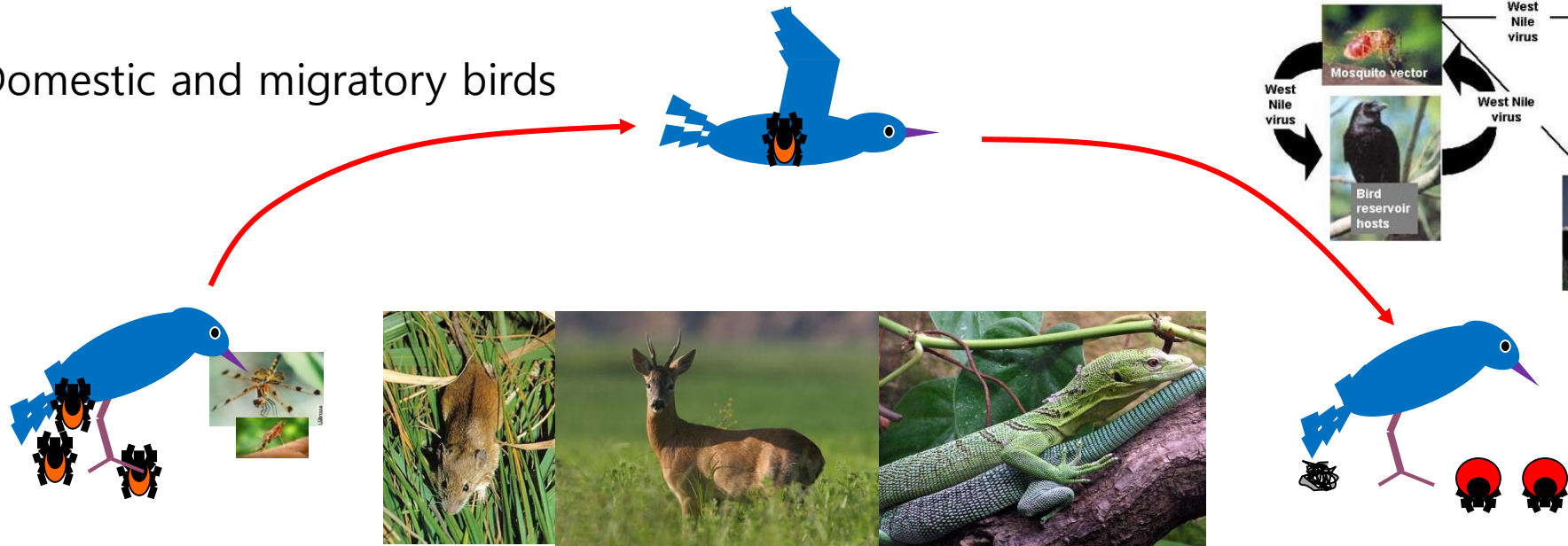
Trewartha : Subtropical zone
 - Monthly mean temperature: above 10°C and above 8 months
 - Minimum temperature of month: below 18°C

Climate and environmental changes

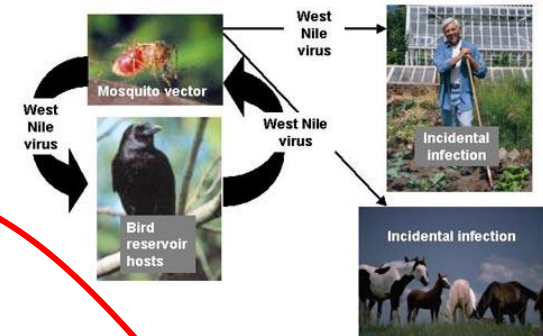


Disease transmission

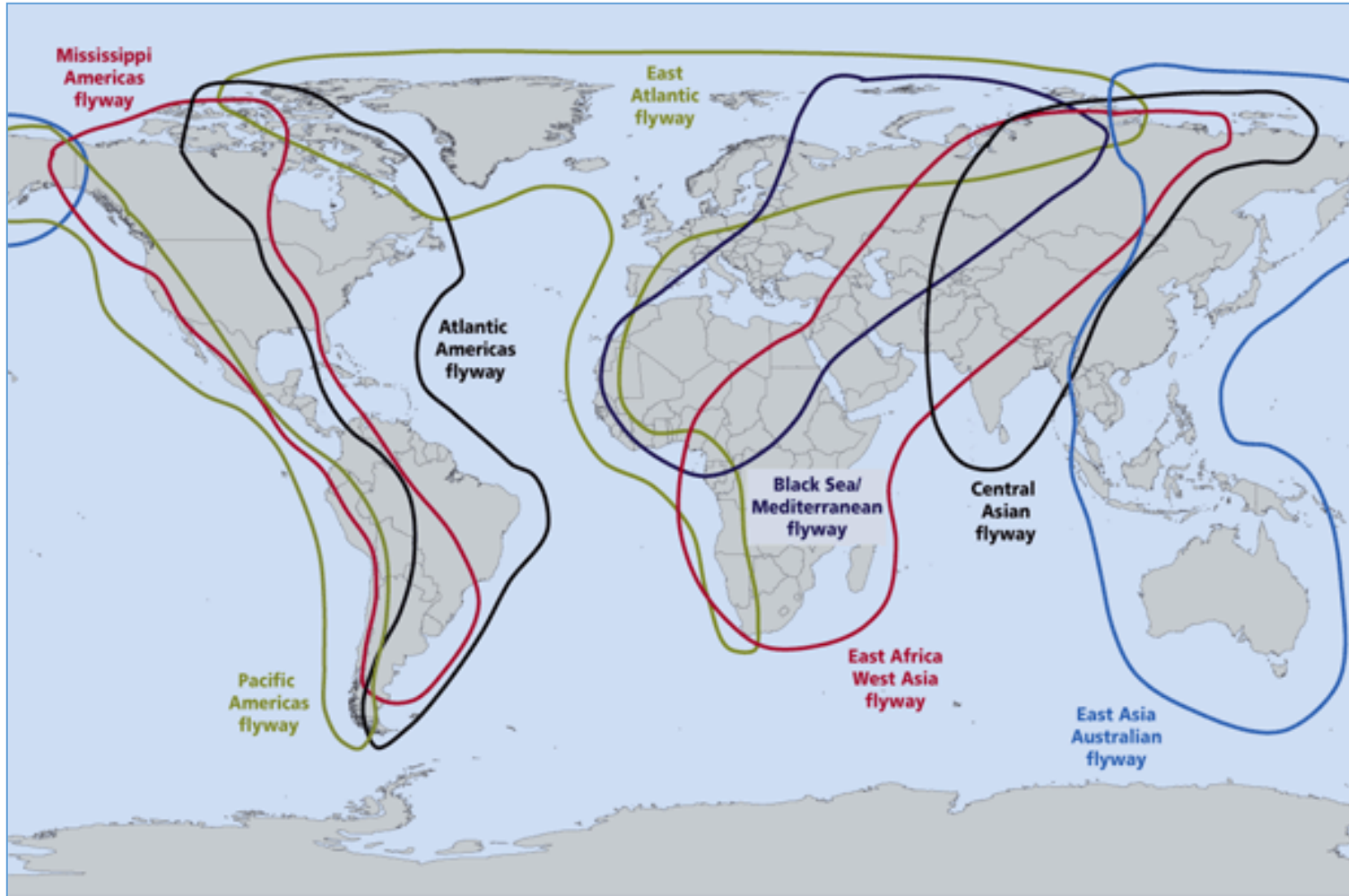
- Life cycle/Food chain
- Insects
 - ✓ Mosquito, ticks, mites, fleas, flies, etc
- Small and large wild mammals
 - ✓ Rodents, Deer, Reptiles, etc
- Birds
 - ✓ Domestic and migratory birds



West Nile Virus Transmission Cycle



Map of route for migratory birds



Stopover sites



Hong Island

- Small area (6.47km²)
- High diversity
- Birds in Korea : 500 species
- Birds in Hong Island : 337 species (data from 2003 – 2007)

Global burden of major vector-borne diseases

Disease	Million DALYs ^a
Mosquito-borne infections	
Malaria	46.5
Lymphatic filariasis	5.8
Dengue	0.62
Japanese encephalitis	0.71
Others^b	
Onchocerciasis	0.48
Leishmaniasis	2.1
African trypanosomiasis	1.5
Chagas disease	0.67

DALY = disability-adjusted life year. ^a Total of DALYs for these diseases represent 17 percent of the global disease burden due to parasitic and infectious diseases. ^b Synanthropic* flies play a major role in the transmission of trachoma and diarrhoeal diseases, but the attributable burden is not readily estimated; other arboviruses and typhus organisms may be of major public health significance but accurate data are not available. *Animals that live in close association with humans (Montana State University Entomology Group, 2007). SOURCE: Reprinted from Townson et al. (2005) with permission from the World Health Organization (<https://health2016.globalchange.gov/>)

Summary of Reported Case Counts of Notifiable Vector-Borne Diseases in the United States

Diseases	2013 Reported Cases	Median (range) 2004–2013
Tick-Borne : about 46,000 cases		
Lyme disease	36,307	30,495 (19,804–38,468)
Spotted Fever Rickettsia	3,359	2,255 (1,713–4,470)
Anaplasmosis/Ehrlichiosis	4,551	2,187 (875–4,551)
Babesiosis	1,792	1,128 (940–1,792)
Tularemia	203	136 (93–203)
Powassan	15	7 (1–16)
Mosquito-Borne : about 5,000 cases		
West Nile virus	2,469	1,913 (712–5,673)
Malaria	1,594	1,484 (1,255–1,773)
Dengue	843	624 (254–843)
California serogroup viruses	112	78 (55–137)
Eastern equine encephalitis	8	7 (4–21)
St. Louis encephalitis	1	10 (1–13)
Flea-Borne		
Plague	4	4 (2–17)

Reported Ixodida ticks in Korea

(2 Family 8 Genus 35 species)

Family Ixodidae(참진드기 科) : hard tick(6속 30종)

- Amblyomma testudinarium* Koch, 1844 뭇뚝참진드기
- Boophilus annuiatus* (Say, 1821) 소참진드기
- B. micropius* (Canestrini, 1887) 꼬리소참진드기
- Dermacentor marginatus* Schulze 광대참진드기
- D. retucuiatus* (fabricius, 1794) 그물무늬광대참진드기
- D. silvarum* Olenev, 1931 은색광대참진드기
- Haemaphysalis campanulata* Warburton, 1908 작은개피참진드기
- H. concinna* Koch, 1844 매부리엉에참진드기
- H. cornigera* Neumann, 1897 들쥐피참진드기
- H. flava* Neumann, 1897 개피참진드기
- H. formosensis***
- H. japonensis* Warburton, 1908 사슴피참진드기
- H. japonica douglasi* Nutt & Warburton, 1908 한뼉엉에참진드기
- H. kutchensis* Hoogstral & Trapdo, 1963 꿩피참진드기
- H. longicornis* Neumann, 1901 작은소참진드기**
- H. ornithophila* Hoogstraal & Kohls, 1959
- H. phasiana* Saito et al, 1974
- Ixodes acuinatus* Neumann 1902 뽕족참진드기
- I. cavipaius* Nuttall & Warburton 고양이참진드기
- I. angustus* Neumann, 1899
- I. granulatus* Supino, 1897 남방참진드기
- I. nipponensis* Kiaoka & Siato, 1967 일본참진드기**
- I. ovatus* Neumann, 1899 사슴참진드기
- I. persulcatus* (Schulze, 1930) 산림참진드기
- I. pomeranzevi* Serdyukova, 1941
- I. signatus* Birula, 1895 새참진드기
- I. simplex***
- I. turdus* Nakatsuji, 1942 고슴도치참진드기
- I. vespertilionis* Koch, 1844 박쥐참진드기
- Rhipcephalus sanguineus* (Latreille, 1806) 개참진드기(뿔진드기)

Family Argasidae (물렁진드기 科) : soft tick

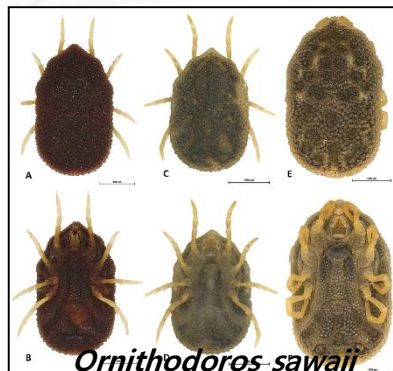
- Argas boueti* Rouband & Colas-Becour, 1933 물렁진드기
- A. japonicus* Yamaguti et al., 1968 일본물렁진드기
- A. vespertilionis* (Latreille, 1802) 둥근박쥐진드기
- Ornithodoros sawaii* Kitaoka and Suzuki (2015년 보고)**
- O. carpensis***

Family Nuttalliellidae

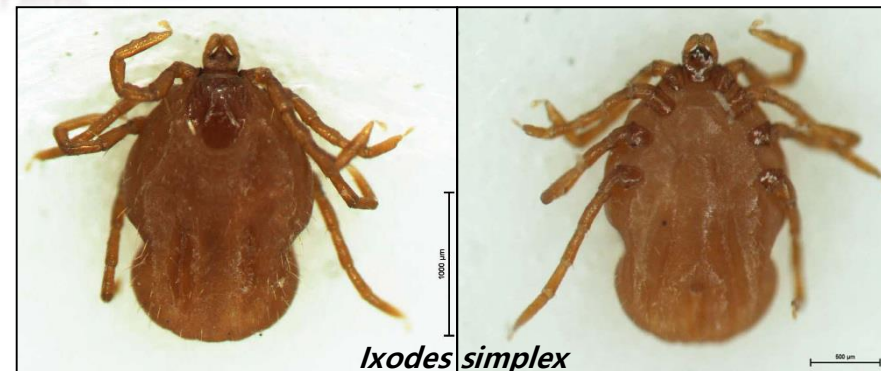
None



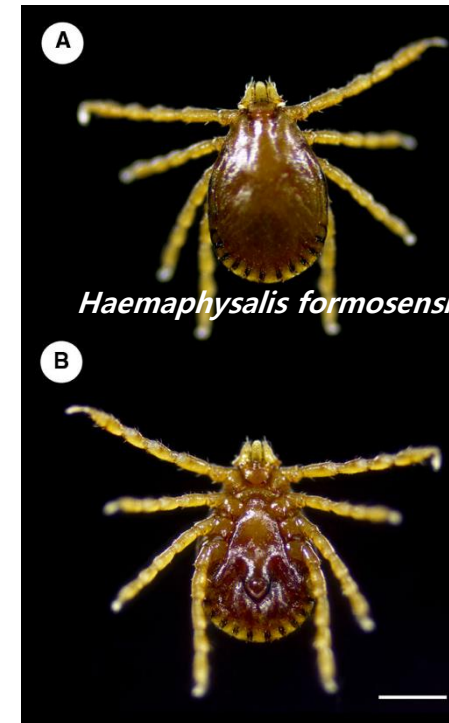
Ixodes and Haemaphysalis spp.



Ornithodoros sawaii



Ixodes simplex



Haemaphysalis formosensis

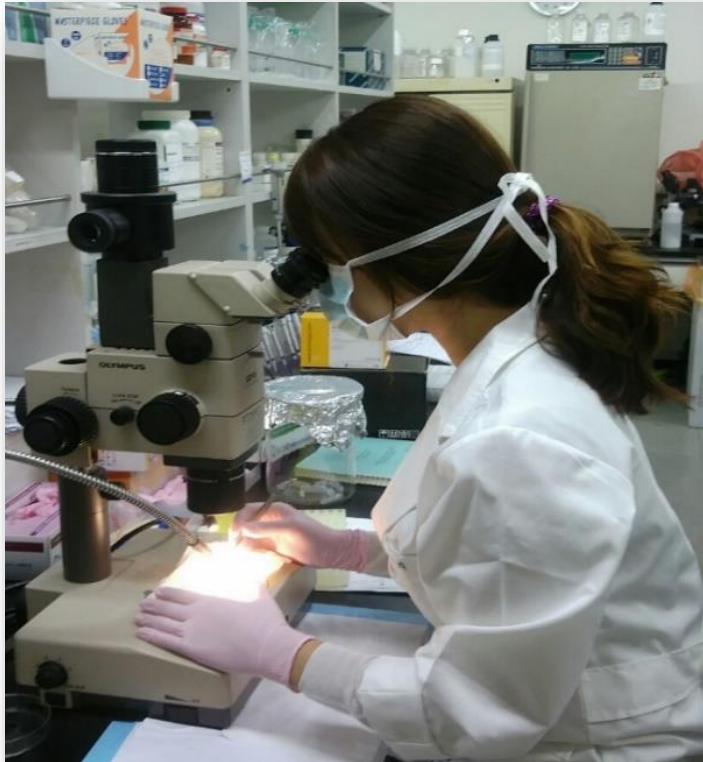
Total number of ticks, by species, collected by tick drag and from small and large mammals, migratory birds, and bats from 2004-2016

Tick species	Rodents	Drag	Birds	Mammals	Bats
<i>Haemaphysalis longicornis</i>		29,429	21	500	
<i>Haemaphysalis flava</i>	12	14,964	52	262	
<i>H. formosensis</i> * (2012년도)		1 (Mara-do)			
<i>Haemaphysalis japonica</i>		13		1	
<i>Haemaphysalis phasiana</i> *		216			
<i>Haemaphysalis ornithophila</i> *			2		
<i>Ixodes granulatus</i>	7				
<i>Ixodes nipponensis</i>	6,748	1,837	28	71	
<i>Ixodes persulcatus</i>		7		42	
<i>Ixodes ovatus</i> (2016)		1 (Deogyusan)			
<i>Ixodes pomerantzevi</i> *	6			44	
<i>Ixodes simplex</i> *					4
<i>Ixodes turdus</i>		17	147		
<i>Ixodes vespertilionis</i>					3
<i>Amblyomma testudinarium</i>		26			
TOTAL (15 species)	6,773	46,509	250	920	7

* New record

Tick collection and identification

- Flagging / Dragging methods
- Stereo microscope



Main tick species in Korea

- Recent collected tick species?

- *Haemaphysalis longicornis*
- *Haemaphysalis flava*
- *Ixodes nipponensis*
- *Amblyomma testudinarium*

Very rare species



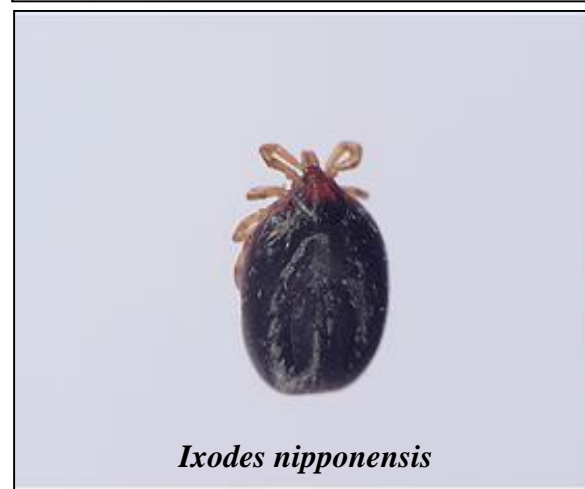
Ixodes ovatus (Deogyusan)



Haemaphysalis longicornis



Haemaphysalis flava



Ixodes nipponensis



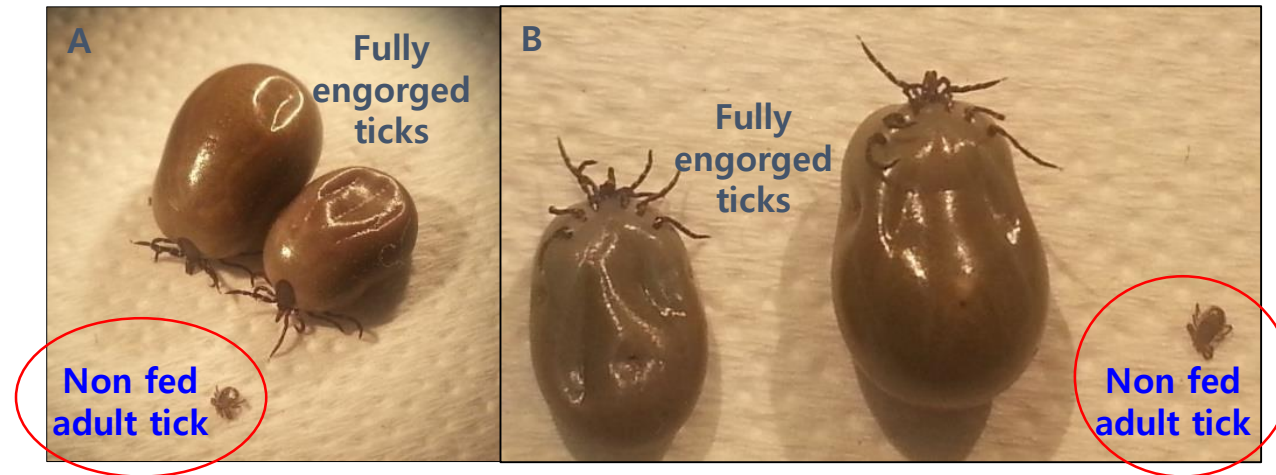
Amblyomma testudinarium

Fully engorged tick species

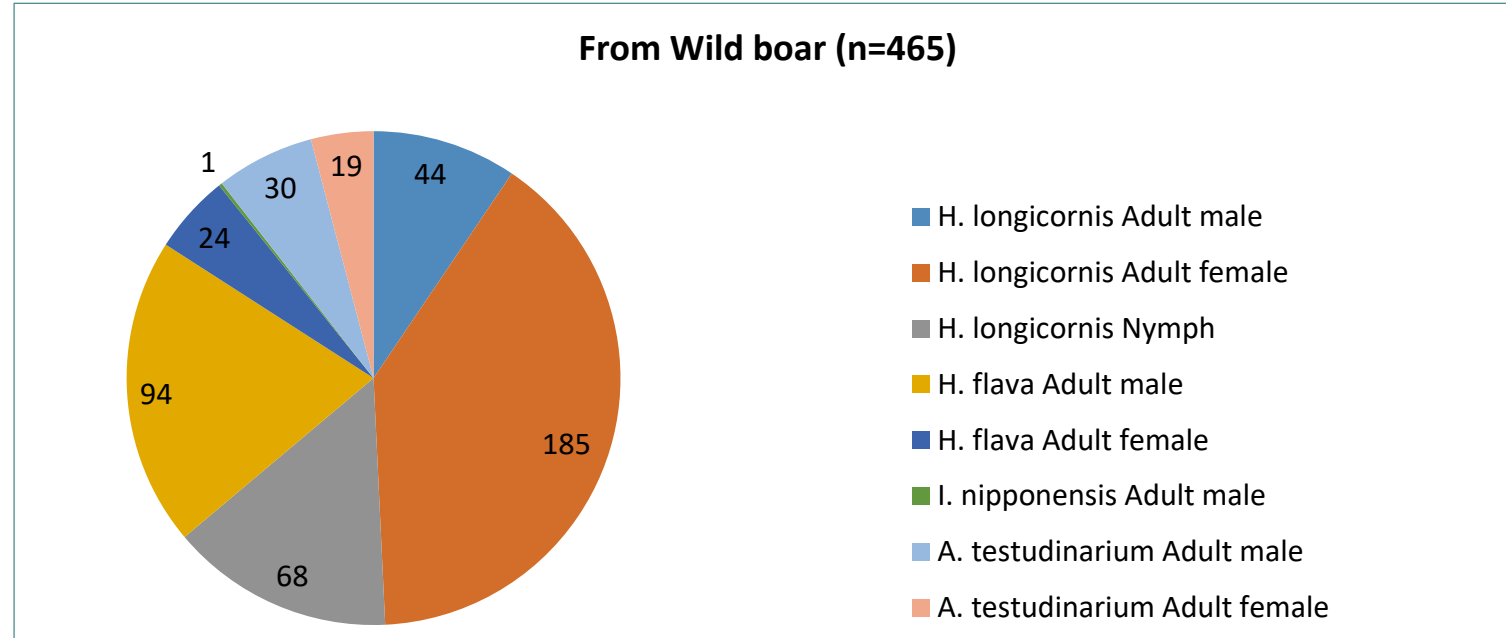
Haemaphysalis longicornis



Ixodes nipponensis



Ixodid ticks from wild boars



Amblyomma testudinarium

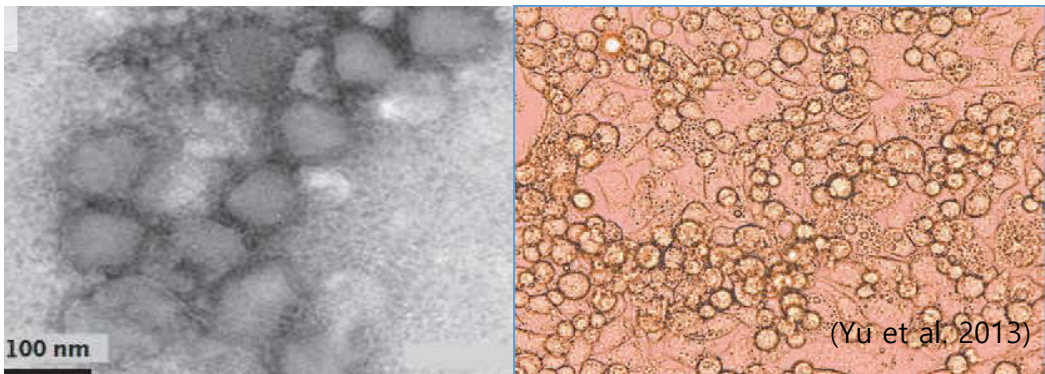
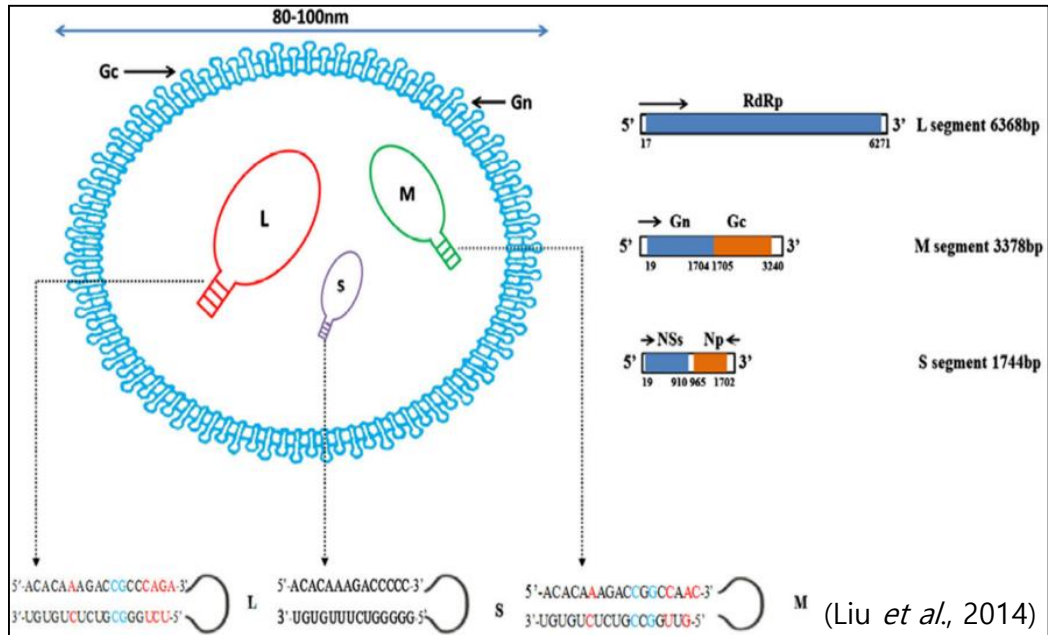


SFTS virus

- Severe fever with thrombocytopenia syndrome virus (SFTSV)
- Tick vector
- In 2017, Family *Bunyaviridae*, re-classification of virus from International Committee on Taxonomy of Viruses (ICRV)
- Bunyavirus : new development and virus isolation
- Phylogenetic analyses by accumulated molecular biological data

Genus	Species
<i>Goukovirus</i>	<i>Cumuto goukovirus</i>
	<i>Gouleako goukovirus*</i>
	<i>Yichang insect goukovirus</i>
<i>Phasivirus</i>	<i>Badu phasivirus*</i>
	<i>Phasi Charoen-like phasivirus</i>
	<i>Wuhan fly phasivirus</i>
	<i>Wutai mosquito phasivirus</i>
Phlebovirus	<i>Bujaru phlebovirus</i>
	<i>Candiru phlebovirus</i>
	<i>Chilibre phlebovirus</i>
	<i>Frijoles phlebovirus</i>
	<i>Punta Toro phlebovirus</i>
	<i>Rift Valley fever phlebovirus</i>
	<i>Salehabad phlebovirus</i>
	<i>Sandfly fever Naples phlebovirus</i>
	SFTS phlebovirus
	<i>Uukuniemi phlebovirus</i>
<i>Tenuivirus</i>	<i>Echinochloa hoja blanca tenuivirus</i>
	<i>Iranian wheat stripe tenuivirus</i>
	<i>Maize stripe tenuivirus</i>
	<i>Rice grassy stunt tenuivirus</i>
	<i>Rice hoja blanca tenuivirus</i>
	<i>Rice stripe tenuivirus</i>
<i>Urochloa hoja blanca tenuivirus</i>	

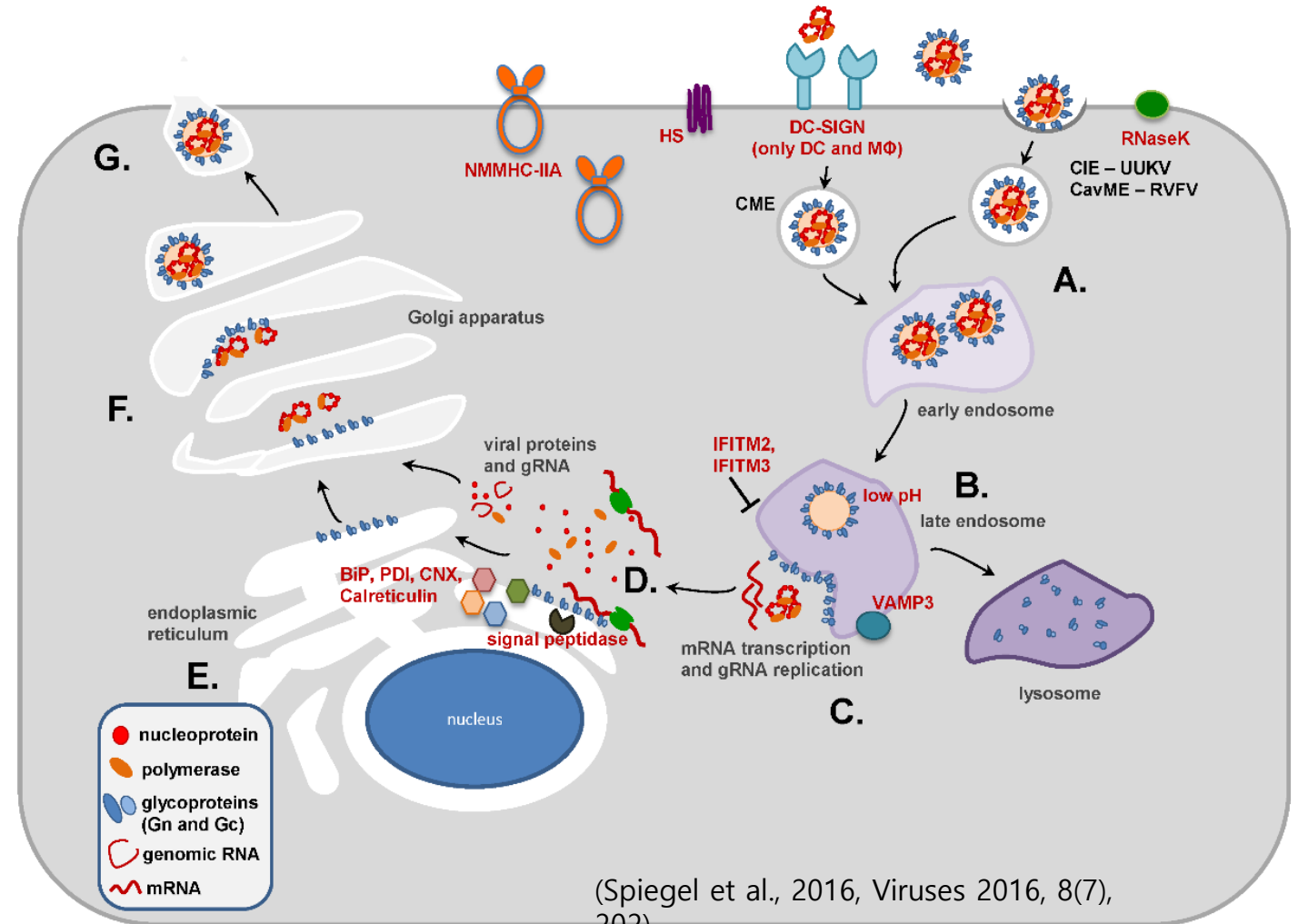
SFTS Phlebovirus



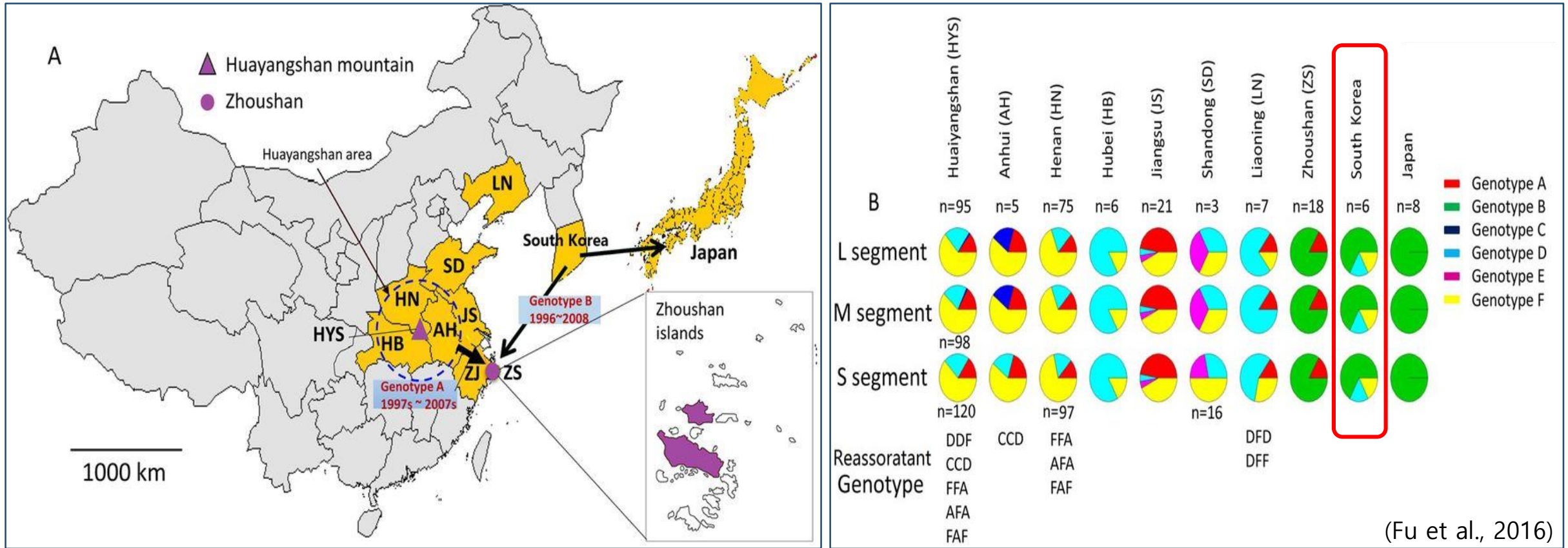
- Virus classification : ICRV (International Committee on Taxonomy of Viruses, 2017)
 - ✓ Group V : (-)ssRNA virus
 - ✓ Order : *Bunyvirales*,
 - ✓ Family : *Phenuiviridae*,
 - ✓ Genus : *Phlebovirus*,
 - ✓ Species : *SFTS Phlebovirus*
- 3 RNA segments (Size **11,490 bp**)
 - ✓ L (large) segment 6,368 bp
 - ✓ M (medium) segment 3,378 bp
 - ✓ S (small) segment 1,744 bp
- DDx : *Anaplasmosis* (*A. phagocytophilum*), hemorrhagic fever with renal syndrome (*Hantavirus*), Scrub typhus (*Orientia tsutsugamushi*), etc.

Replication cycle of *Phlebovirus*

- C57BL/6 mouse model
- SFTSV RNA detection
 - ✓ Blood, spleen, liver, kidney
 - ✓ virus proliferation in spleen
 - ✓ Virus attaches platelets
 - ✓ Phagocytosis by macrophage in the spleen
 - ✓ Thrombocytopenia



Distribution of SFTS virus



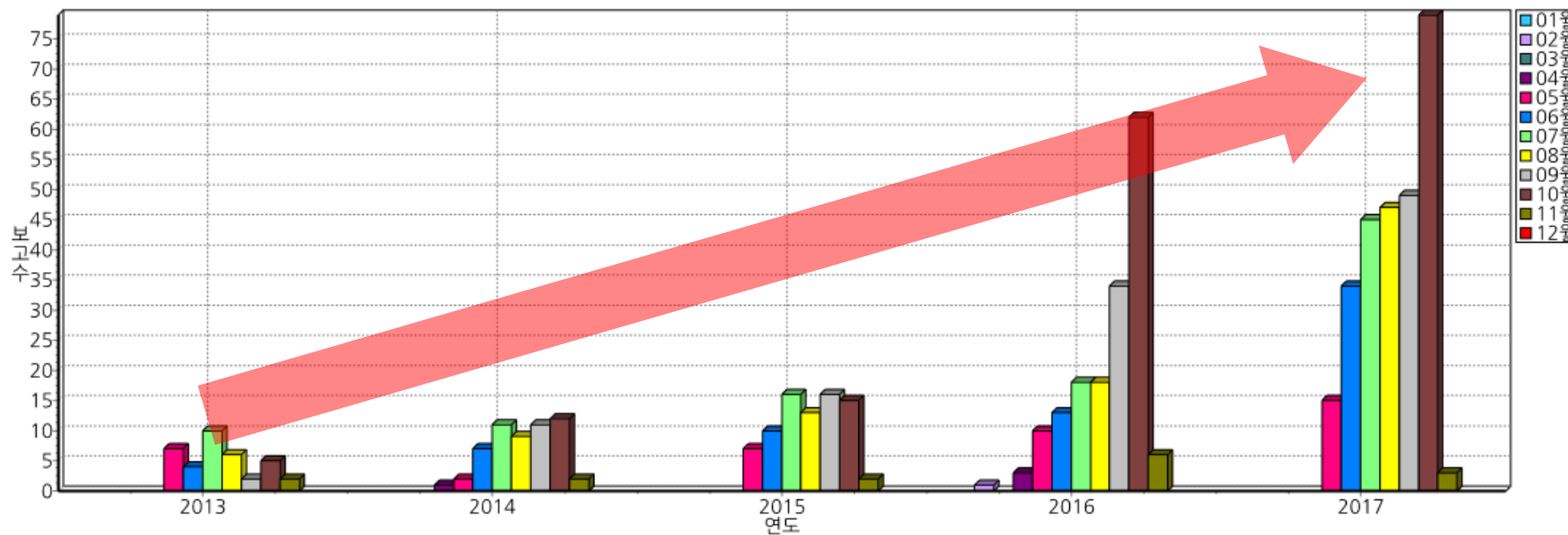
SFTS virus in Korea

- First confirmed case from human in Korea
- Increase number of patients

Mortality	47.2%	29.1%	26.5%	11.5%	19.8 % (12월 말)
Patients	36명	55명	79명	165명	272명 (12월 말)
No. of death	17명	16명	21명	19명	54 명 (12월 말)

152 (Aug)
(759)

Total
20.9%
607명
127명



2018

Detection of SFTS antigen and antibody in animals

Percentage of sero-prevalence and RNA detection in animal models

	Antibody detection	RNA detection	Reference
Sheep	69.5	-	[29]
Chicken	47.4	-	[29]
Goat	74.8	-	[29]
Cattle	2.2-60.5	1.7-5.3	[29,30,33]
Goat	66.8	-	[29,30,31]
Dog	0.23-55	-	[24,29,34,35]
Pig	2-6	-	[29,30,34]
Cat	0.46	-	[35]
Korean water deer	23.81	4.76	[37]
Wild boar	1.85-51	3.7	[37,38]
Hedgehogs	14	5.4	[37]
Common shrew	-	2	[37]
Fulvous harvest	-	2.2	[37]
Rodent	4.4-11.3	-	[36,39-42]

Animal models for SFTSV infection

Animal	Strain	Nonlethal/lethal	Reference
Mouse	Ifih1 ^{tm1.1Cln}	Nonlethal	Not published
	IFNAR ^{-/-} 129/Sv	Lethal	[50-52]
	IFNAR ^{-/-} C57BL/6	Lethal	[52,53]
	BALB/c adult	Nonlethal	[52]
	C57BL/6 adult	Nonlethal	[52]
	C57BL/6 (mytociin treated)	Lethal	[52]
	FVB/NJ adult	Nonlethal	[52]
New borne (KM, BALB/c, C57BL/6)		Lethal	[52]
		Nonlethal	[52]
Hamster		Nonlethal	[52]
Rat	Wistar	Nonlethal	[52]
	Newborn	Lethal	[52]
Macaque	Rhesus Macaque	Nonlethal	[54]

SFTSV, severe fever and thrombocytopenia syndrome virus.

(Robles et al., 2018, Clin Exp Vaccine Res 7:43-50)

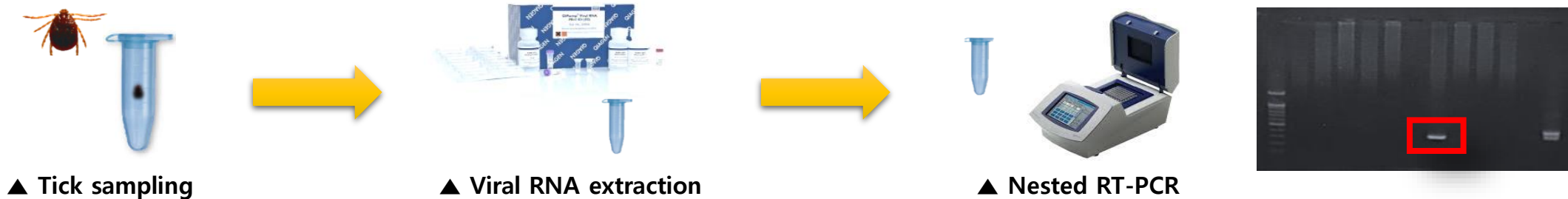
Detection of SFTS virus

- Amplification of specific gene particle
- Nested reverse transcriptase (RT)-PCR
- Detection of the **SFTSV S segment (346bp)**
- Amplicons were sequenced

Contents	Primers	sequences
One-step RT-PCR(1 st round PCR) primers	SFTSV NP-2F	5'-CAT CAT TGT CTT TGC CCT GA-3' (20mer)
	SFTSV NP-2R	5'-AGA AGA CAG AGT TCA CAG CA-3' (20mer)
One-step RT-nested PCR (2nd round PCR) primers	SFTSV N2-F	5'-AA ^Y AAG ATC GTC AAG GCA TCA-3' (21mer)
	SFTSV N2-R	5'-TAG TCT TGG TGA AGG CAT CTT-3' (21mer)

(Y : C or T)

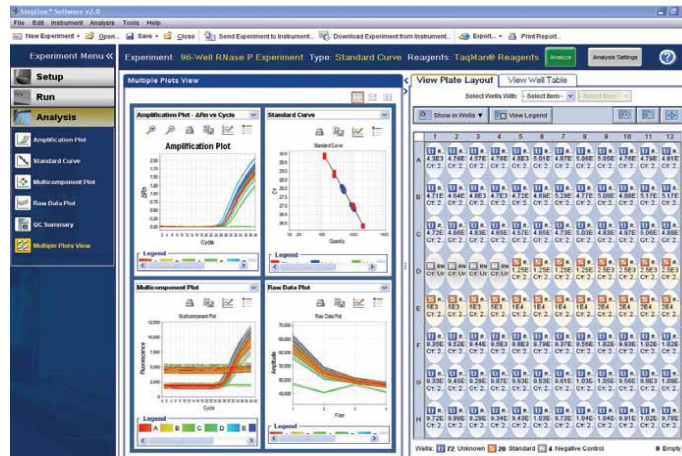
Modified primer sequences (Shimajima et al. 2013)



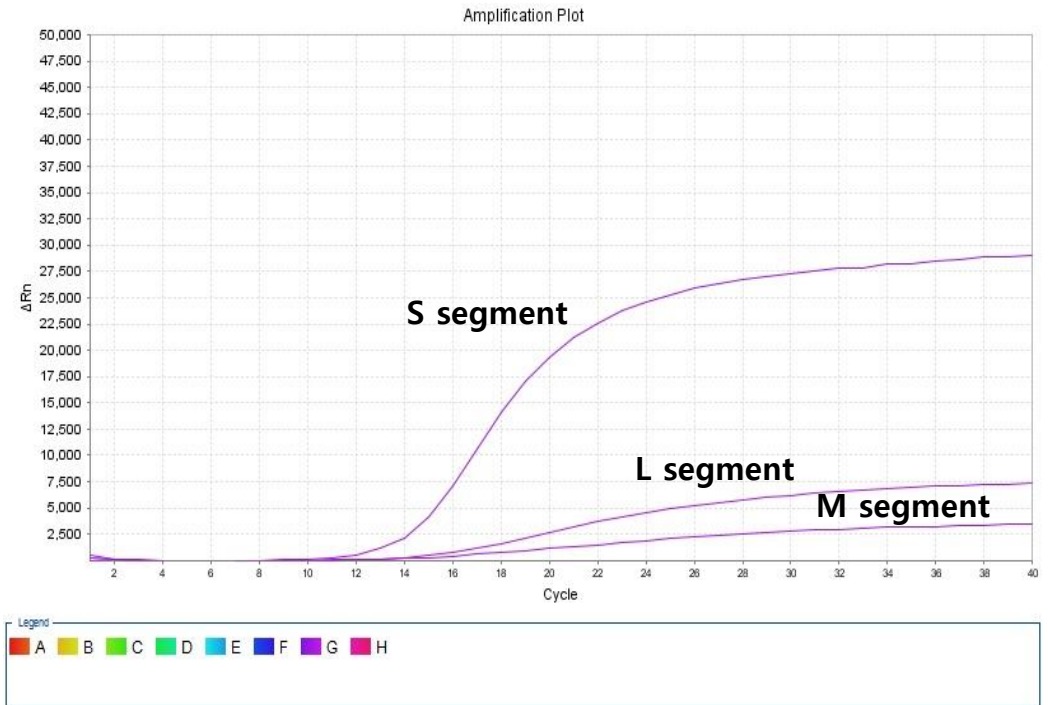
Real-time PCR method

- Establishment of SFTS virus detection method by Real-time PCR
 - ✓ TaqMan real-time PCR (qPCR)
 - ✓ Setup for PCR primers and conditions
- Comparison between One-Step RT/Nested-PCR and RT real-time PCR methods : specificity and sensitivity

- Real-time PCR amplification : SFTSV genome segment (L, M, S)
- S > L > M segment



PCR (SimpliAmp Thermal Cycler, (Applied Biosystems Real-time PCR System)



Detection of SFTS virus from ticks

(Comparison between Real-time and Nested PCR)

- Sample : nymph stage
- Single tick survey

Real-time PCR

Tick collection sites	No. tested ticks	No. positive ticks (Ct <30)	Infection rate (IR, %)
Seokmo-do	471	9	1.91
Ganghwa-do	470	6	1.28
Deogusan	470	13	2.77
Total	1,411	28	1.98

Nested PCR

Tick collection sites	No. tested ticks	No. positive ticks	Infection rate (IR, %)
Seokmo-do	451	46	10.20
Ganghwa-do	419	32	7.64
Deogusan	470	33	7.02
Total	1,340	111	8.28

Comparison between single tick and pooling tick samples by Nested PCR

- Collected ticks (*H. longicornis nymph*) from April to July, 2016 in Seokmo and Ganghwa islands
- Infection rates (IR, 7.12%) : tested individually
- Minimum field infection rates (MFIR, 0.58%): tested by pooled ticks (3 adults, 10 nymphs)

$$\text{Minimum field infection rate (MFIR, \%)} = \frac{\text{Number of positive pools}}{\text{Number of total tested ticks}} \times 100$$

- ✓ Minimum field infection rate (MFIR, %)
- ✓ Number of positive pools
- ✓ Number of total tested ticks

Tick collection sites	No. tested ticks	No. positive ticks	IR (%)	No. tested pools (No. ticks)	No. positive pools	MFIR (%)
Seokmo-do	1,400	109	7.79	97 (959)	7	0.73
Ganghwa-do	399	19	4.76	48 (429)	1	0.23
Total	1,799	128	7.12	145 (1,388)	8	0.58

SFTS virus survey in 5 National Parks (2015)

■ Surveyed National Parks



■ Trail



■ Non-trail



Comparison between trail and non-trail in 5 National Parks (2015)

Mountain	No. of SFTS positive ticks/No. of Collected ticks							
	Trail				Non-trail			
	Adult (IR, %)	Nymph (IR, %)	*Larva (MFIR, %)	Total (MFIR, %)	Adult (IR, %)	Nymph (IR, %)	*Larva (MFIR, %)	Total (MFIR, %)
Bukhansan	2/24 (8.33)	3/35 (8.57)	1/591 (0.17)	6/650 (0.92)	5/58 (8.62)	2/226 (0.88)	6/1,333 (0.45)	13/1,617 (0.80)
Chiaksan	2/31 (6.45)	1/165 (0.61)	0/497 (0.00)	3/693 (0.43)	0/1 (0.00)	1/21 (4.76)	3/991 (0.30)	4/1,013 (0.39)
Deogyusan	0/8 (0.00)	0/25 (0)	0/0 (-)	0/33 (0.00)	4/75 (5.33)	8/219 (3.65)	3/1,030 (0.29)	15/1,324 (1.13)
Juwangsan	9/55 (16.36)	2/49 (4.08)	0/607 (0.00)	11/711 (1.55)	4/58 (6.90)	2/60 (3.33)	9/1,968 (0.46)	15/2,086 (0.72)
Wolchulsan	3/43 (6.98)	3/79 (3.80)	2/952 (0.21)	8/1,074 (0.74)	0/62 (0.00)	3/188 (1.60)	2/315 (0.63)	5/565 (0.88)
Total	16/161 (9.94)	9/353 (2.55)	3/2,647 (0.11)	28/3,161 (0.89)	13/254 (5.12)	16/714 (2.24)	23/5,637 (0.41)	52/6,605 (0.79)

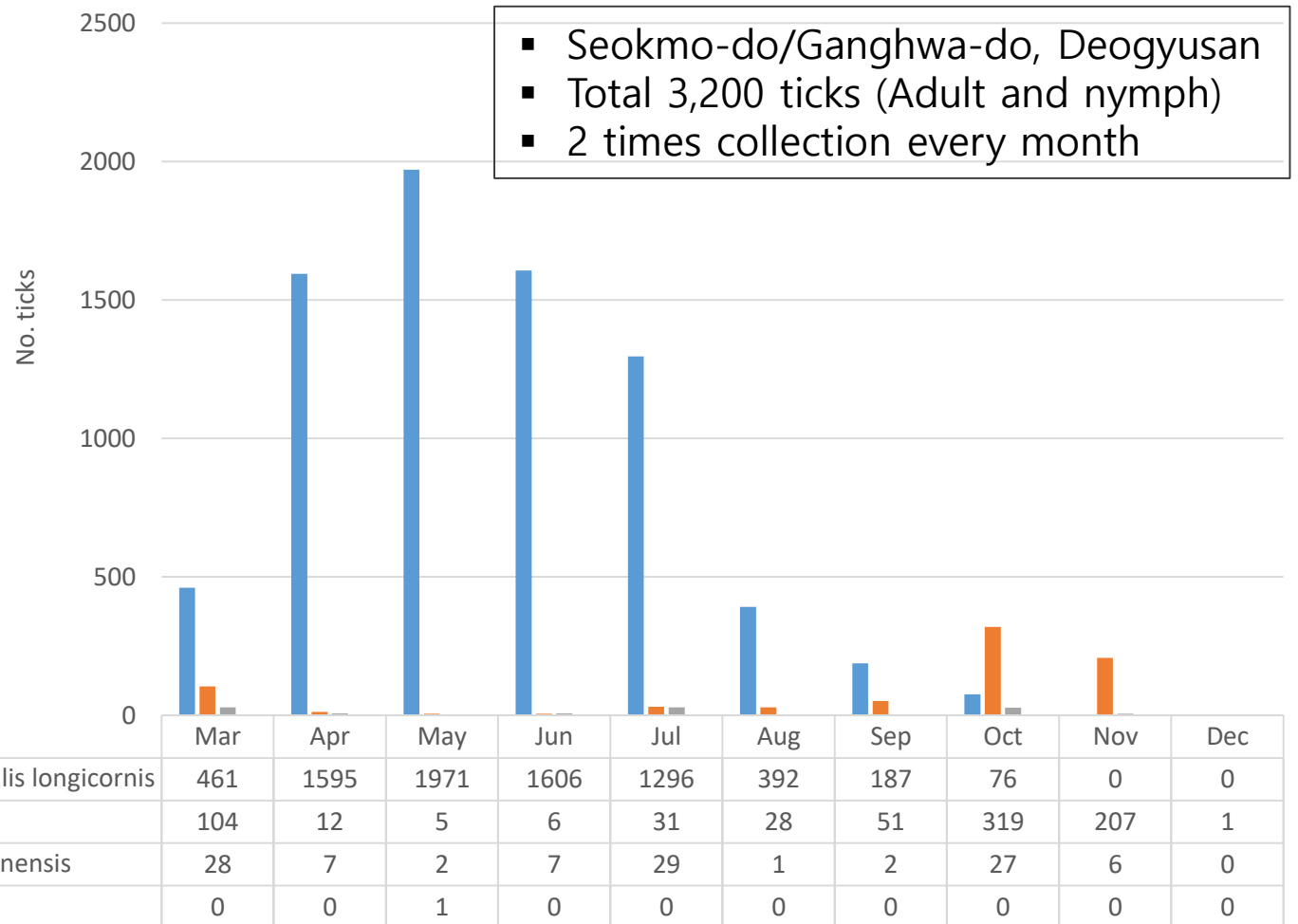
(*pooling tick test)

Detection of SFTS virus from ticks (2015)

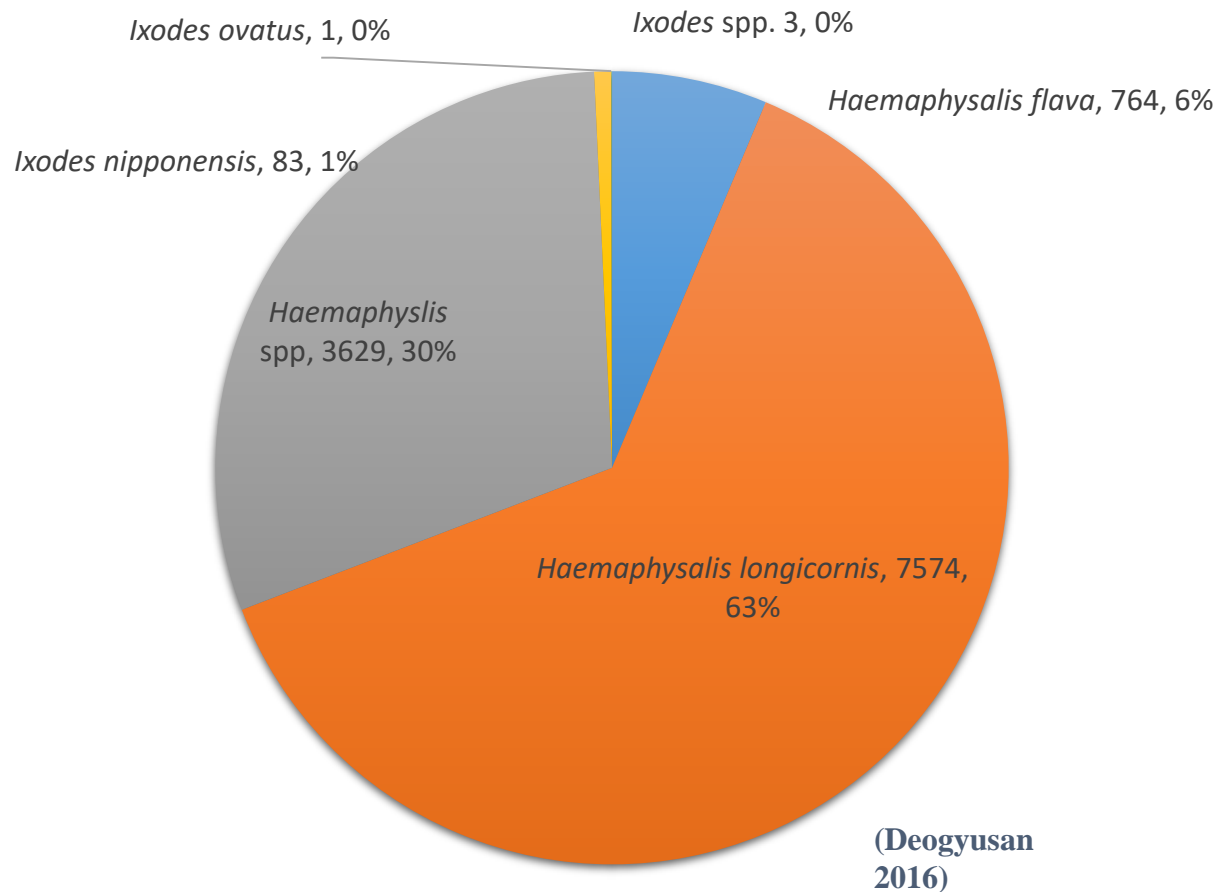
(Single tick test, tick species, 5 National Parks)

Tick species	Developmental stages	No. tested ticks	No. PCR-positive ticks	Infection rates* (%)
<i>Haemaphysalis longicornis</i>	Female	224	21	9.38
	Male	37	2	5.41
	Nymph	745	25	3.36
	Subtotal	1,006	48	4.77
<i>Haemaphysalis flava</i>	Female	56	1	1.79
	Male	77	2	2.60
	Nymph	303	2	0.66
	Subtotal	436	5	1.15
<i>Ixodes nipponensis</i>	Female	5	0	0
	Male	9	0	0
	Nymph	9	0	0
	Subtotal	23	0	0
<i>Amblyomma testudinarium</i>	Male	1	0	0
	Nymph	4	1	25
	Subtotal	5	1	20
Total		1,470	54	3.67

Tick collection in Deogusan National Park (2016)



Tick collection in Deogyusan National Park (2016)



Haemaphysalis longicornis



Haemaphysalis flava



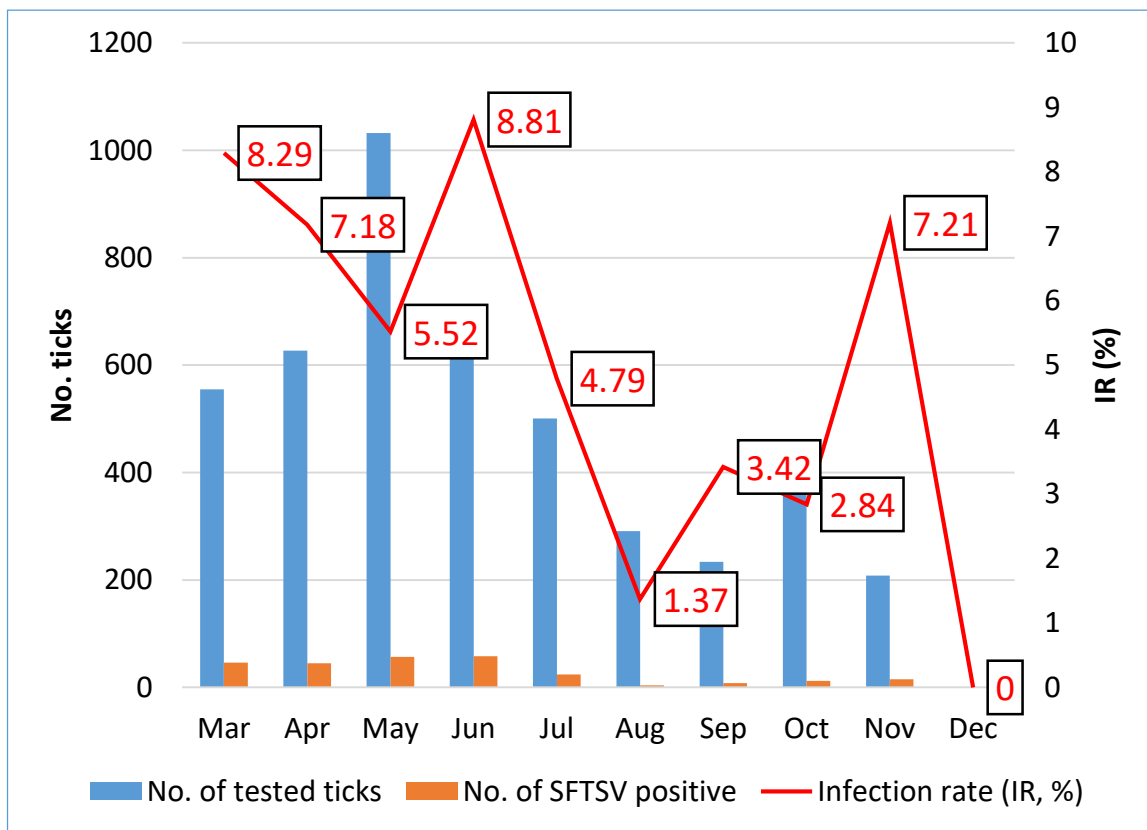
Ixodes nipponensis



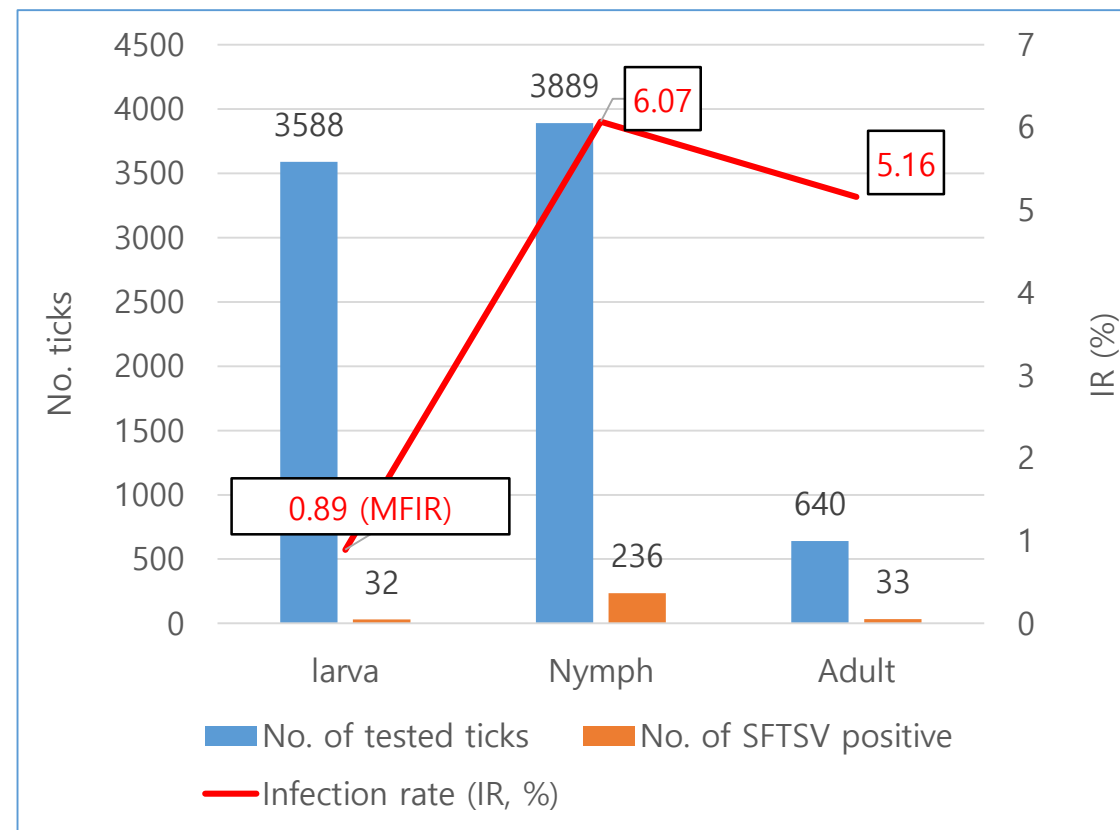
Ixodes ovatus

Infection rates of SFTS virus (Deogusan, 2016)

SFTSV infection rates by monthly

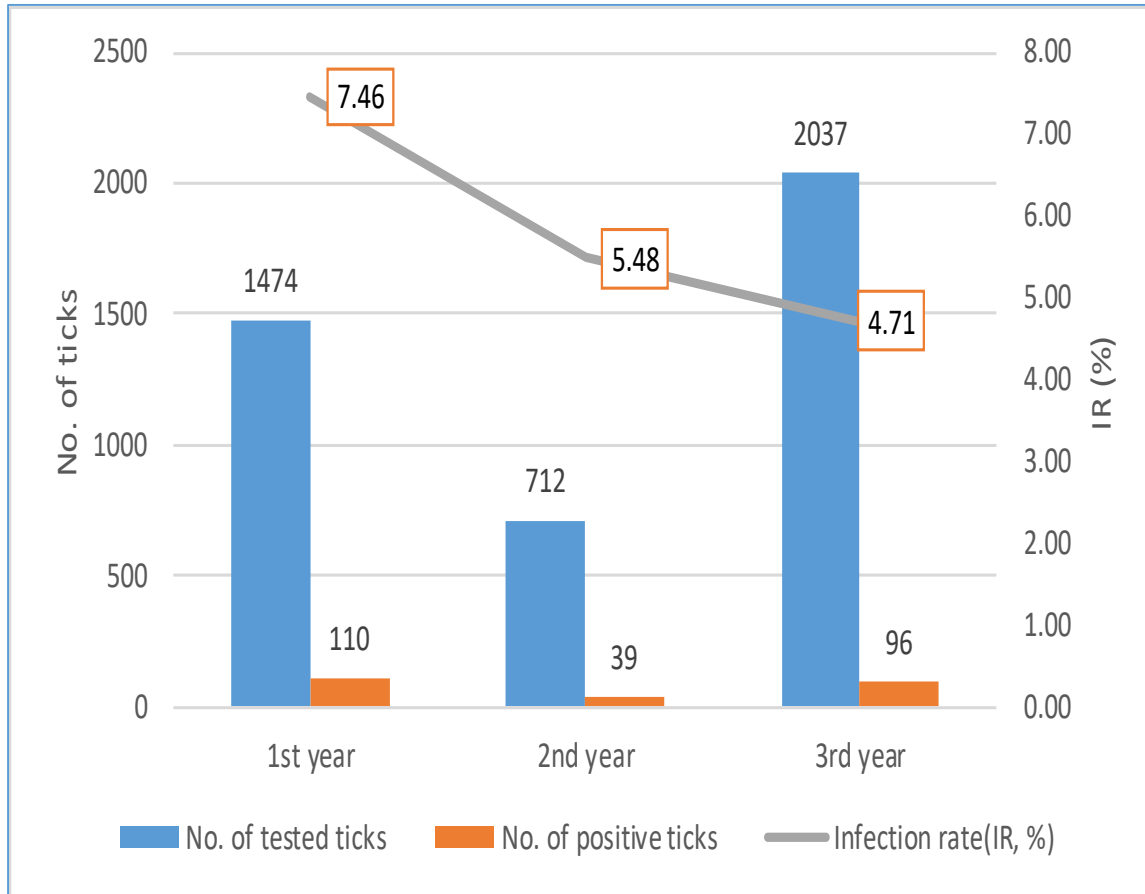


SFTSV infection rates by developmental stages

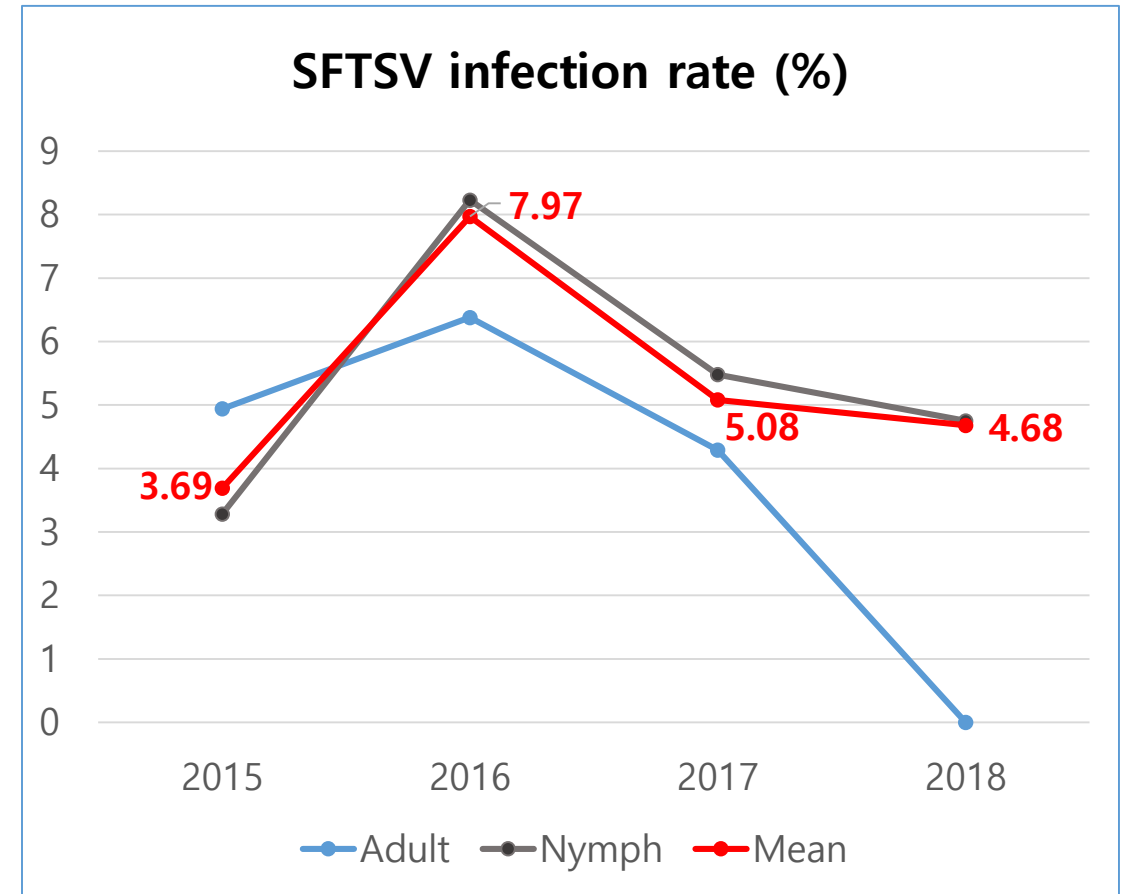


Detection of SFTS virus

(Single tick test, Yearly, Deogusan, 2015~2018)



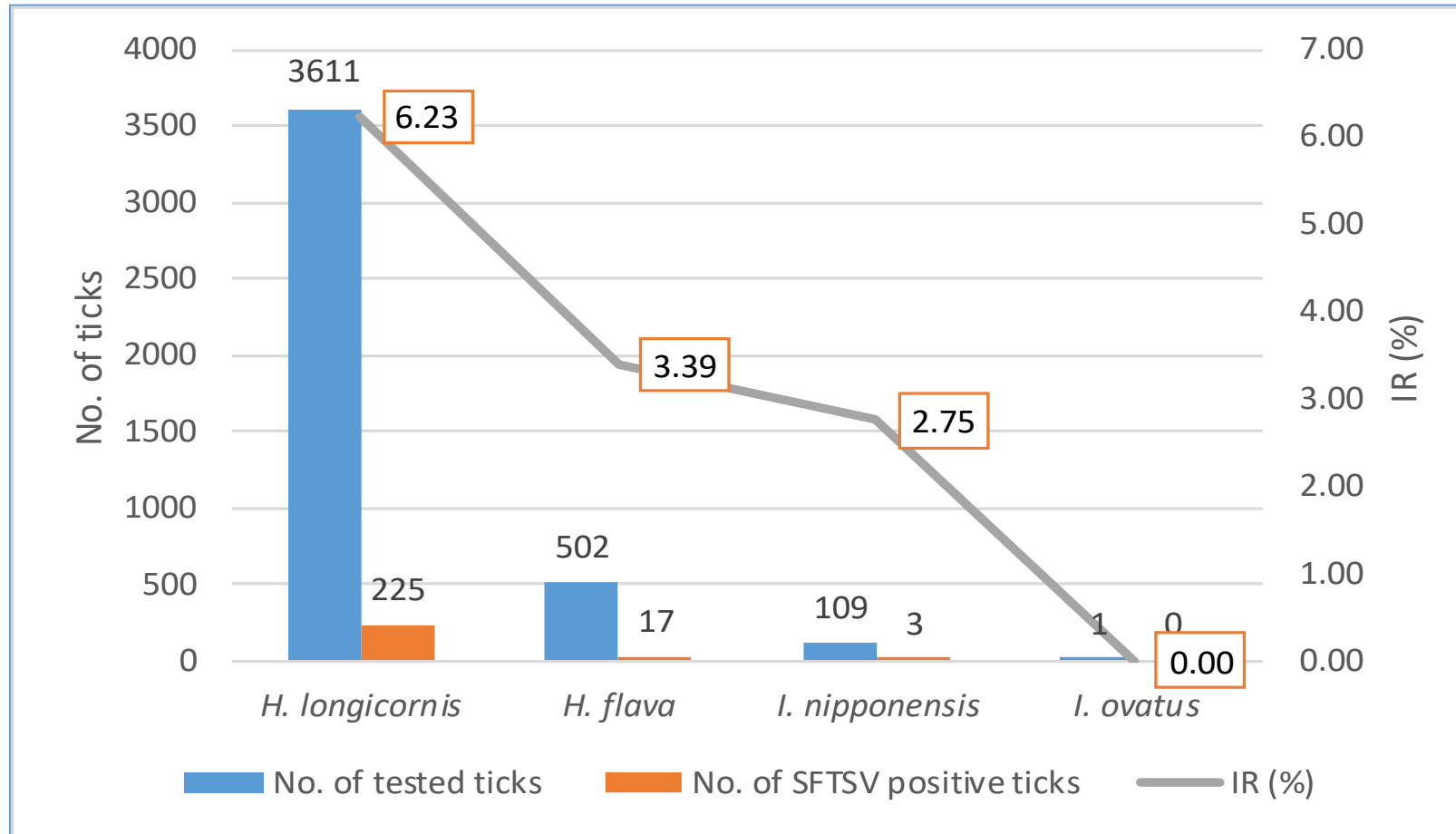
(1st year : 2015. 7~2016. 6; 2nd year: 2016. 7~2017. 6; 3rd year: 2017. 7~2018. 6)



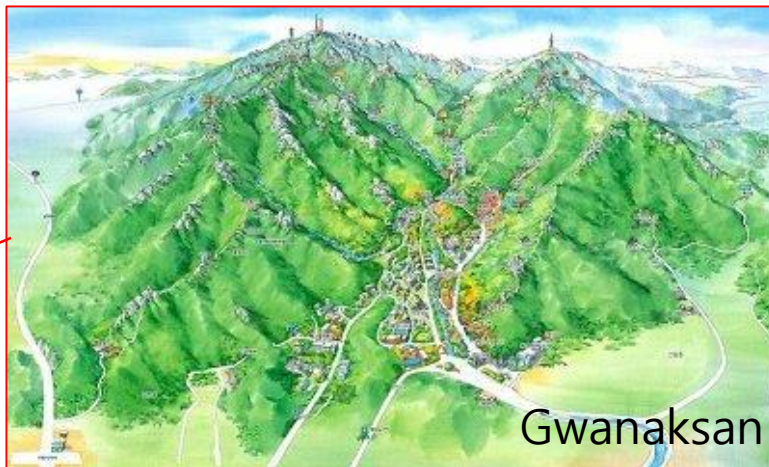
(Data : July, 2015 ~ June, 2018)

Detection of SFTS virus

(Single tick test, Tick species, Deogusan, 2015~2018)



Detection of SFTS virus from ticks on Gwanaksan



Korean J Vet Res(2017) 57(3) : 169~174
<https://doi.org/10.14405/kjvr.2017.57.3.169>

Original article (2015)

Prevalence of severe fever with thrombocytopenia syndrome virus among ticks surveyed at Mt. Gwanak, Korea

Species	Developmental stages	No. of ticks	SFTS positive pools (MFIR, %)
<i>Haemaphysalis longicornis</i>	Adult Female	4	0
	Adult Male	-	0
	Nymph	72	1 (1.39)
	Subtotal	76	1 (1.32)
<i>Haemaphysalis flava</i>	Adult Female	9	0
	Adult Male	3	0
	Nymph	37	3 (8.11)
	Subtotal	49	3 (6.12)
Subtotal	125	4 (3.20)	
<i>Haemaphysalis spp.</i>	Larva	148	3 (2.03)
	Subtotal	148	3 (2.03)
Total		273	7 (2.56)

(Chae et al., 2017. Korean J Vet Res(2017) 57(3) : 169~174)

Detection of SFTS virus from domestic pigs

K-1-5/2013. 8. 27

Pig White Female 90 kg Pohang, Gyeongsangbuk-do



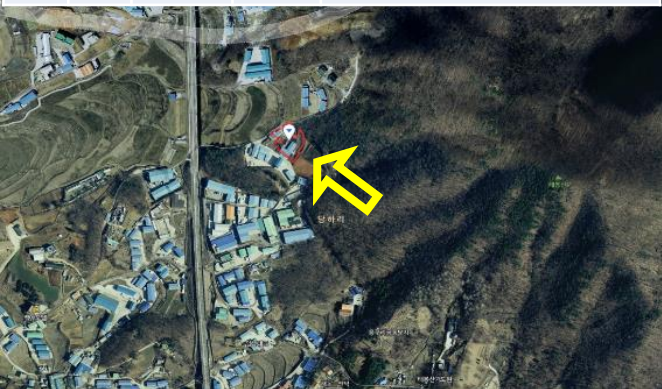
S-1-1/2013. 9. 2

Pig Black Female 70 kg Changnyeong, Gyeongsangnam-do



G-6-1/2013. 9. 24

Pig Black Female 120 kg Hwaseong, Gyeonggi-do



K-4-5/2013. 9. 25

Pig White Female 80 kg Yeongdeog, Gyeongsangbuk-do

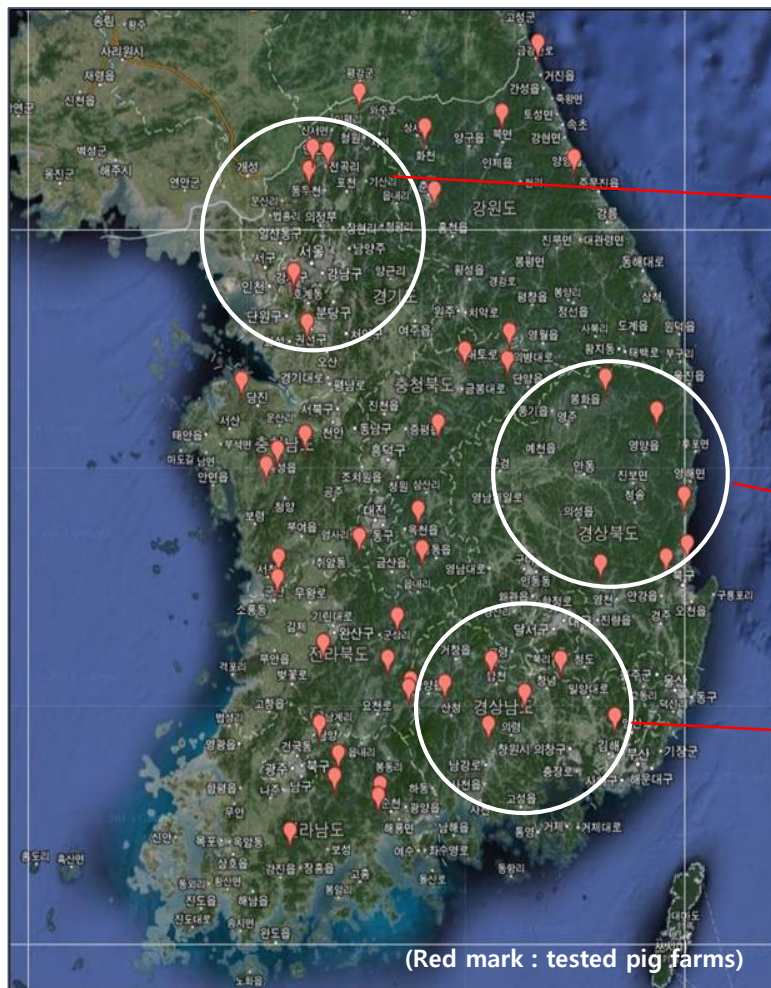


Near by the mountain

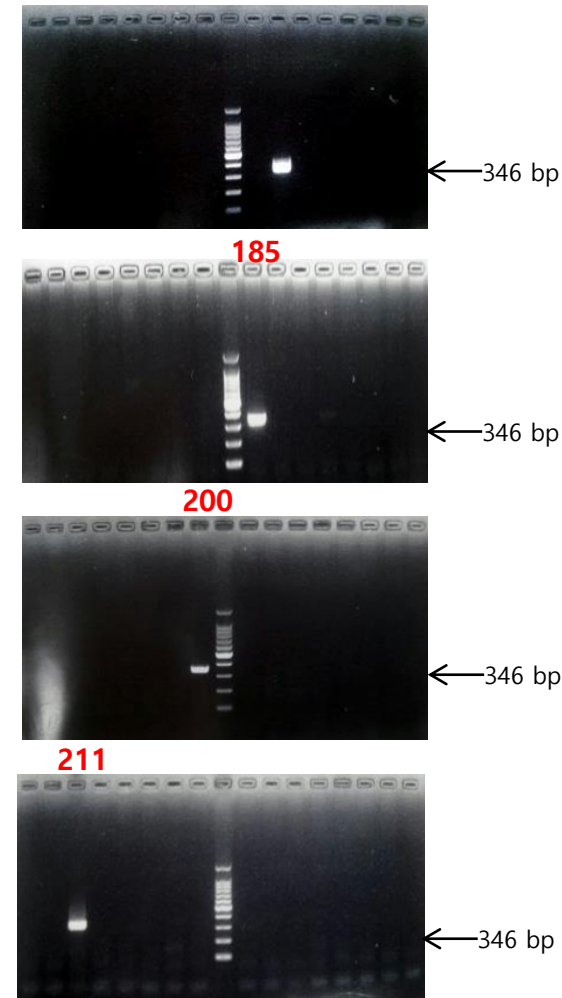
Traditionally reared pigs



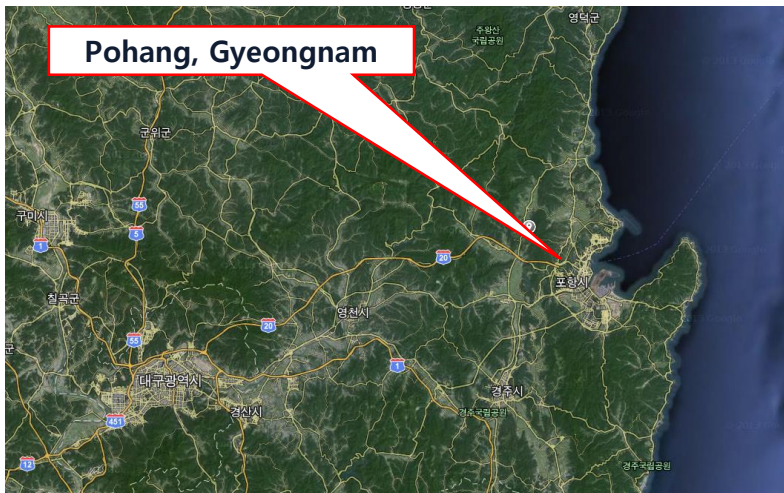
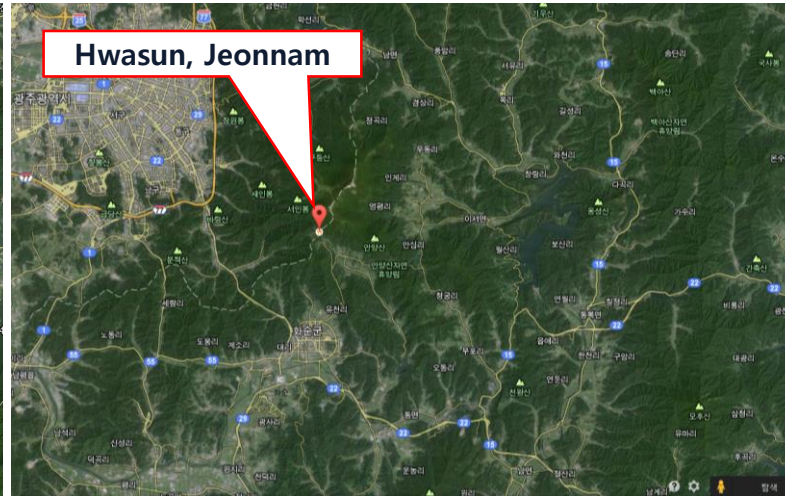
Detection of SFTS virus from domestic pigs



Provinces	Pigs	No. of pigs	Total	SFTS virus	Infection rates
Gangwon-do	White	5	30	0	0
	Black	15			
	Boar	10			
Gyeonggi-do	White	10	30	1	3.33
	Black	20			
Chungcheongbuk-do	Black	30	30	0	0
Chungcheongnam-do	White	5	30	0	0
	Black	20			
	Boar	5			
Jeollabuk-do	White	20	30	0	0
	Black	10			
Jeollanam-do	White	12	30	0	0
	Black	18			
Gyeongsangbuk-do	White	15	30	2	6.67
	Black	15			
Gyeongsangnam-do	White	15	30	1	3.33
	Black	10			
	Boar	5			
Total	White	82	240	4	1.67
	Black	138			
	Boar	20			



Detection of SFTS virus from **black goat**



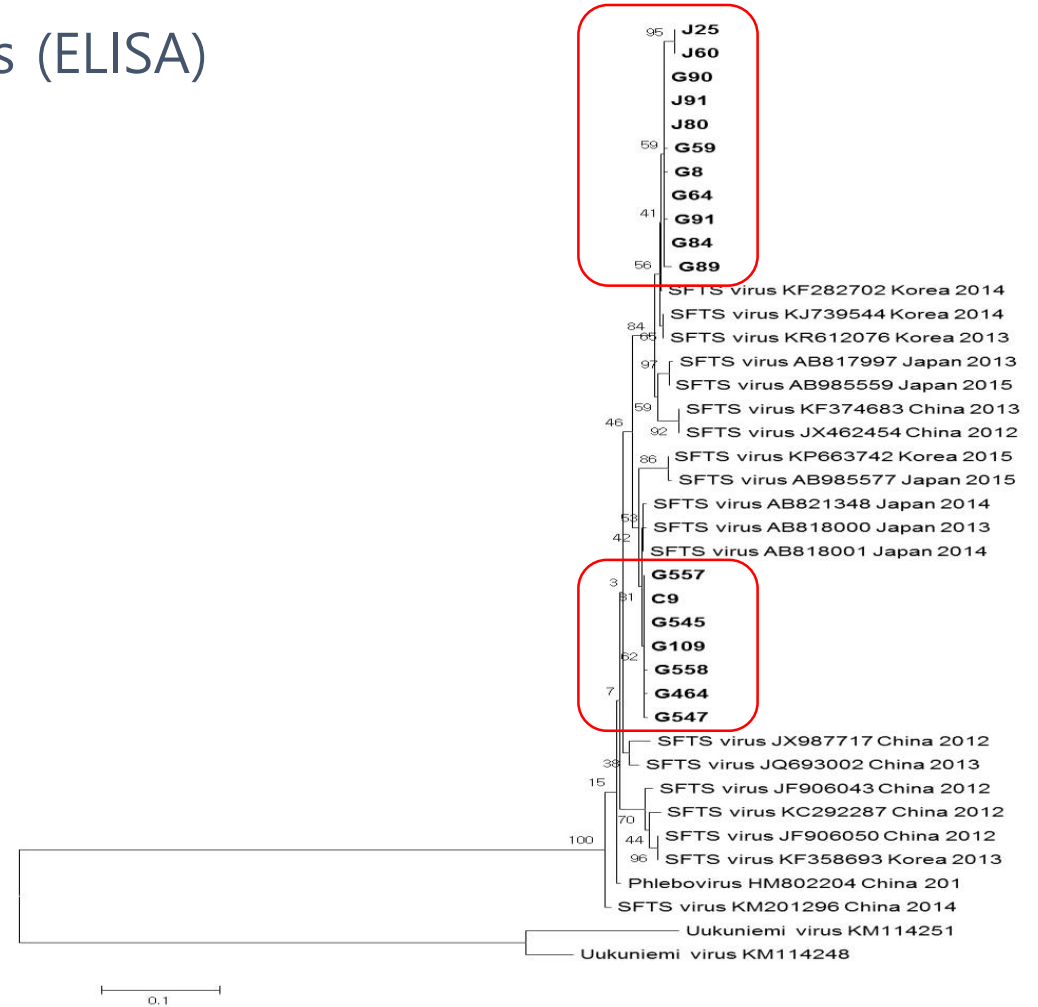
▪ Ranches for black goat (2015)

Area	No. of serum	Positive for SFTSV	Infection rates (%)
Namwon, Jeonbuk	100	5	5.0
Hwasun, Jeonnam	99	8	8.1
Pohang, Gyeongnam	30	0	0
Namwon, Jeju	39	1	2.6
Total	268	14	5.2

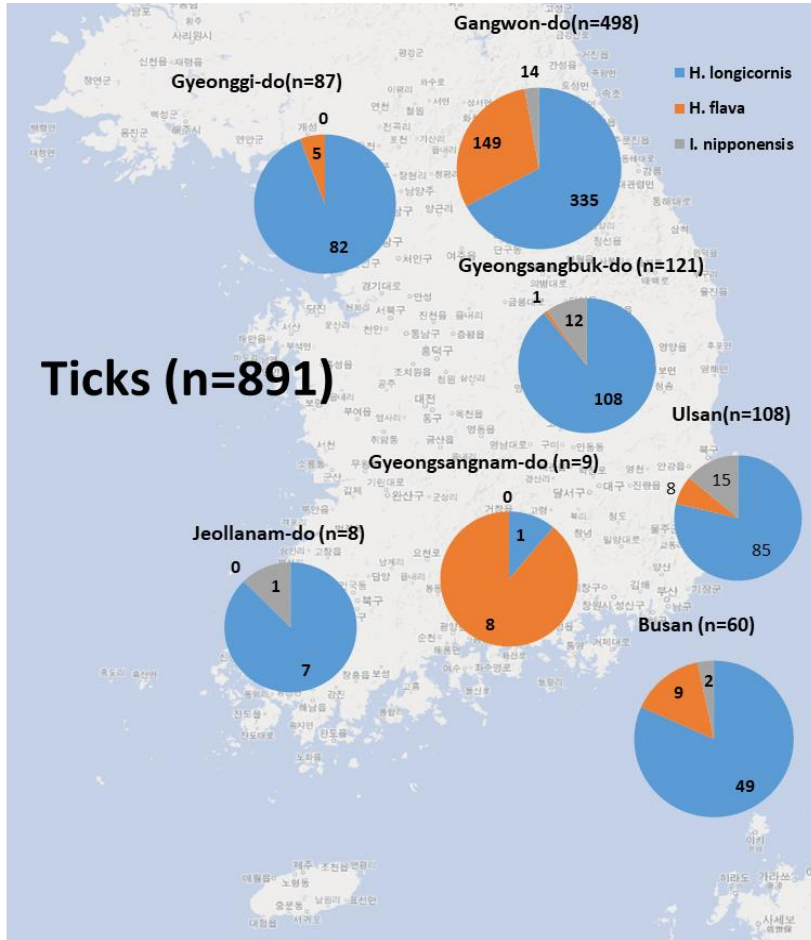
Detection of SFTS virus from black goat

- ELISA test : 637 sera out of 737 sera samples (ELISA)
- Blood sample from slaughter house (2015)**

Region	No. of samples	No. of PCR positive (%)	No. of ELISA positive
Gangwon	20	0	3 (15.0)
Gyeonggi	31	2 (6.5)	2 (6.5)
Gyeongnam	25	2 (8.0)	1 (4.0)
Gyeongbuk	13	0	1 (7.7)
Jeonbuk	180	6 (3.3)	5 (6.3)*
Jeonnam	325	6 (1.8)	20 (6.2)
Jeju	4	0	1 (25.0)
Chungbuk	24	0	6 (25.0)
Chungnam	115	2 (1.7)	4 (3.5)
Total	737	18/737 (2.4)	43/637 (6.8)



Detection of SFTS virus in **ticks** collected from **Korean water deer** and **wild boars**

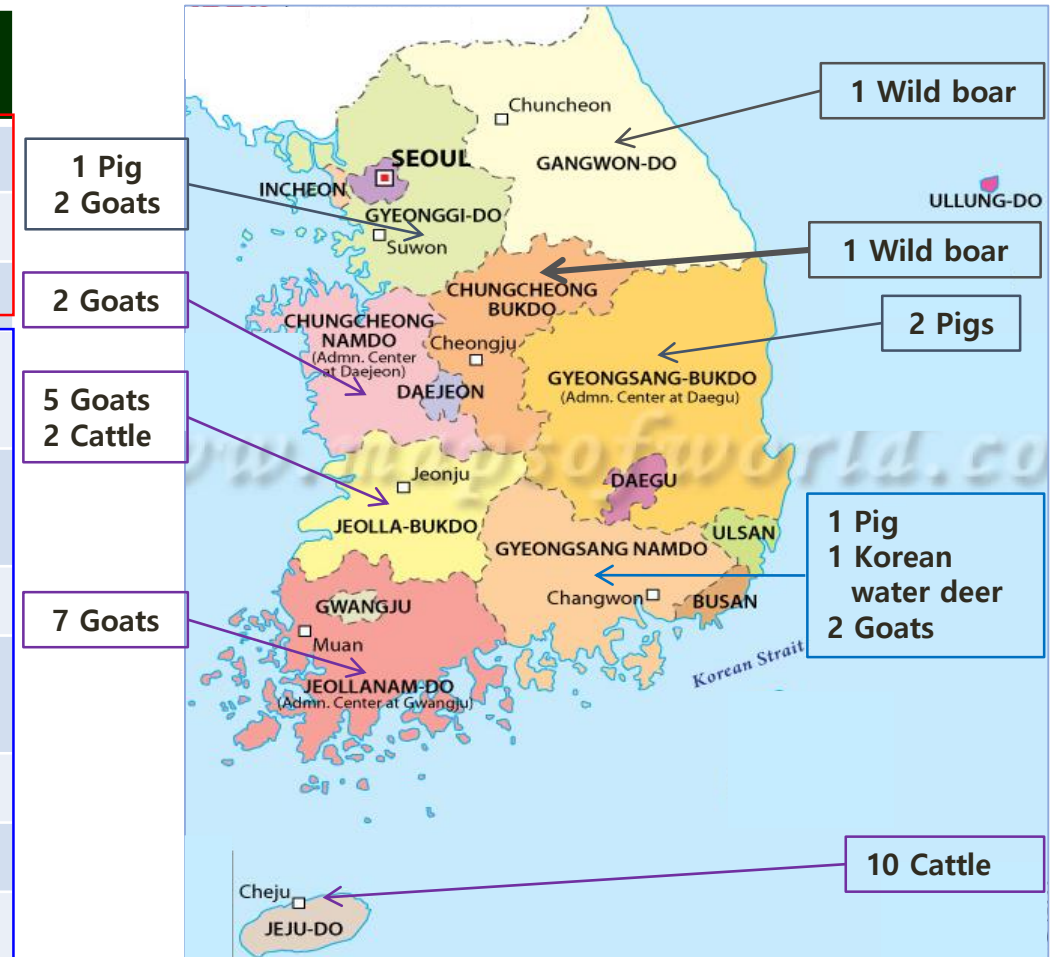


Species	Developmental stages of ticks	No. of analyzed ticks	No. of pools	No. of positive pools	MIR (%)
<i>H. longicornis</i>	Larva	120	17	1 ^a	0.83
	Nymph	156	56	11	7.05
	Adult male	57	24	5	8.77
	Adult female	266	196	10	3.76
	Subtotal	599	293	27	4.51
<i>H. flava</i>	Larva	23	6	0	0
	Nymph	31	9	1	3.23
	Adult male	68	30	2	2.94
	Adult female	58	56	1	1.72
	Subtotal	180	101	4	2.22
<i>I. nipponensis</i>	Larva	3	1	0	0
	Nymph	2	2	1	50.00
	Adult male	13	10	5	38.46
	Adult female	26	26	4	15.38
	Subtotal	44	39	10	22.73
Total	Larva	146	24	1	0.68
	Nymph	189	67	13	6.88
	Adult male	138	64	12	8.70
	Adult female	350	278	15	4.29
	Total	823	433	41	4.98

^aThis pool was fed larvae.
MIR, minimum infection rate.

Detection of SFTS virus from domestic and wild animals

	Animals	No. of Samples	No. of positive	Infection Rates (%)
가축	Domestic pigs	240	4	1.7
	Cattle (from Jeju island)	99	12	12.1
	Goats	737	18	2.4
야생동물	Korean water deer (<i>Hydropotes inermis argyropus</i>)	21	1	4.8
	Siberian roe deer (<i>Capreolus pygargus</i>)	3	0	0
	Goral (<i>Naemorhedus caudatus</i>)	5	0	0
	Raccoon dog (<i>Nyctereutes procyonoides</i>)	7	0	0
	Wild boar (<i>Sus scrofa</i>)	54	2	3.7
	Carrion crow (<i>Corvus corone</i>)	1	0	0
	Total		1,167	37



Detection of SFTS virus from feral cats

Items	Classification	Number of samples	Number of PCR results; No. of positives (%)
Collected regions <p> , 유기묘 포획 지역 , SFTS virus 검출 지역 </p>	Guro-gu	11	1 (9.1%)
	Mapo-gu	8	3 (37.5%)
	Geumcheon-gu	60	9 (15%)
	Seongdong-gu	30	7 (23.3%)
	Yongsan-gu	4	1 (25%)
	Dongdaemoon-gu	12	1 (8.3%)
	Gangnam-gu	1	0 (0%)
	Total number of samples		126

(Hwang et al., Ticks and Tick-borne Diseases 2017:8;9–12)

Comparison of detection rates between **feral** and **house cat**

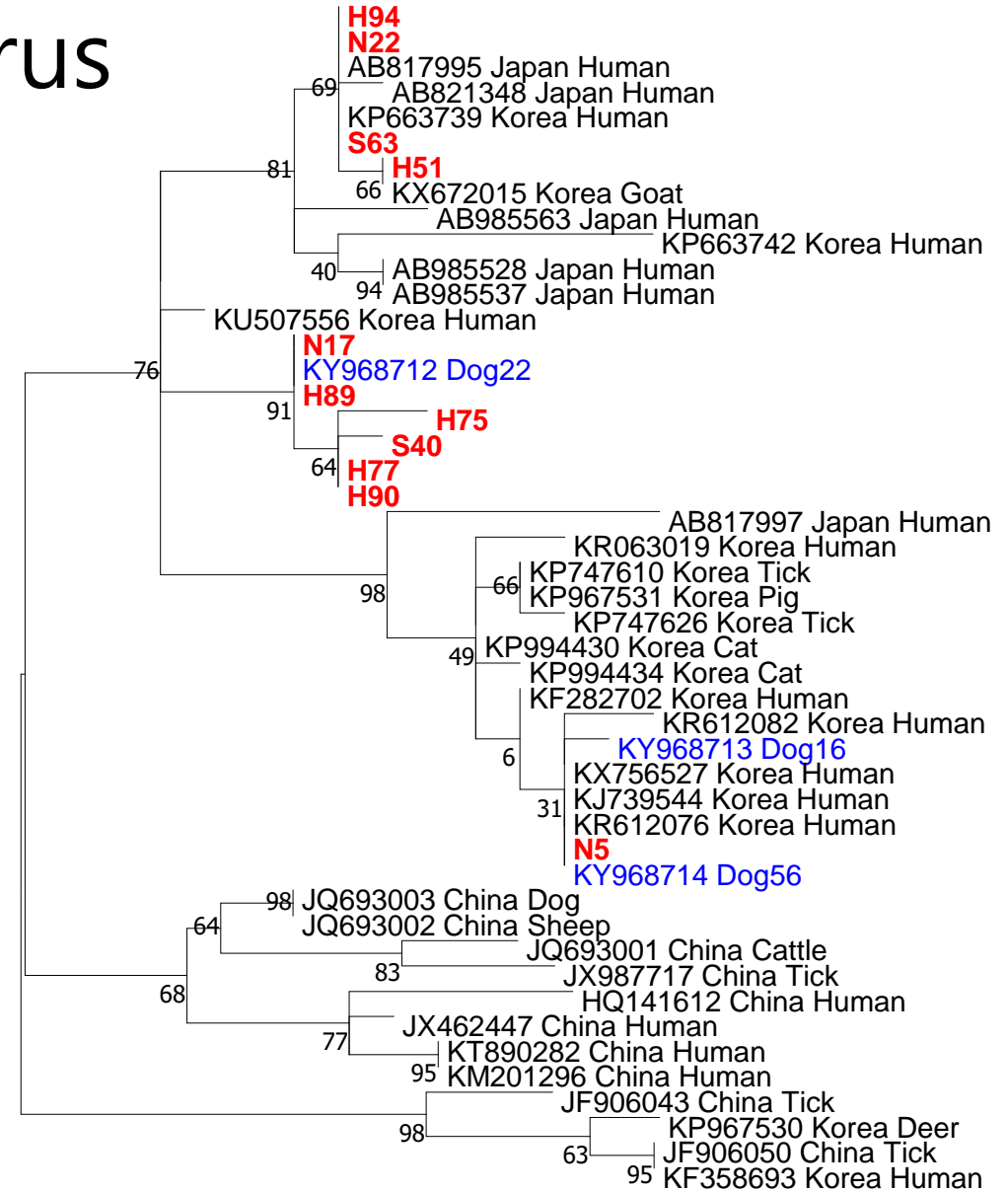
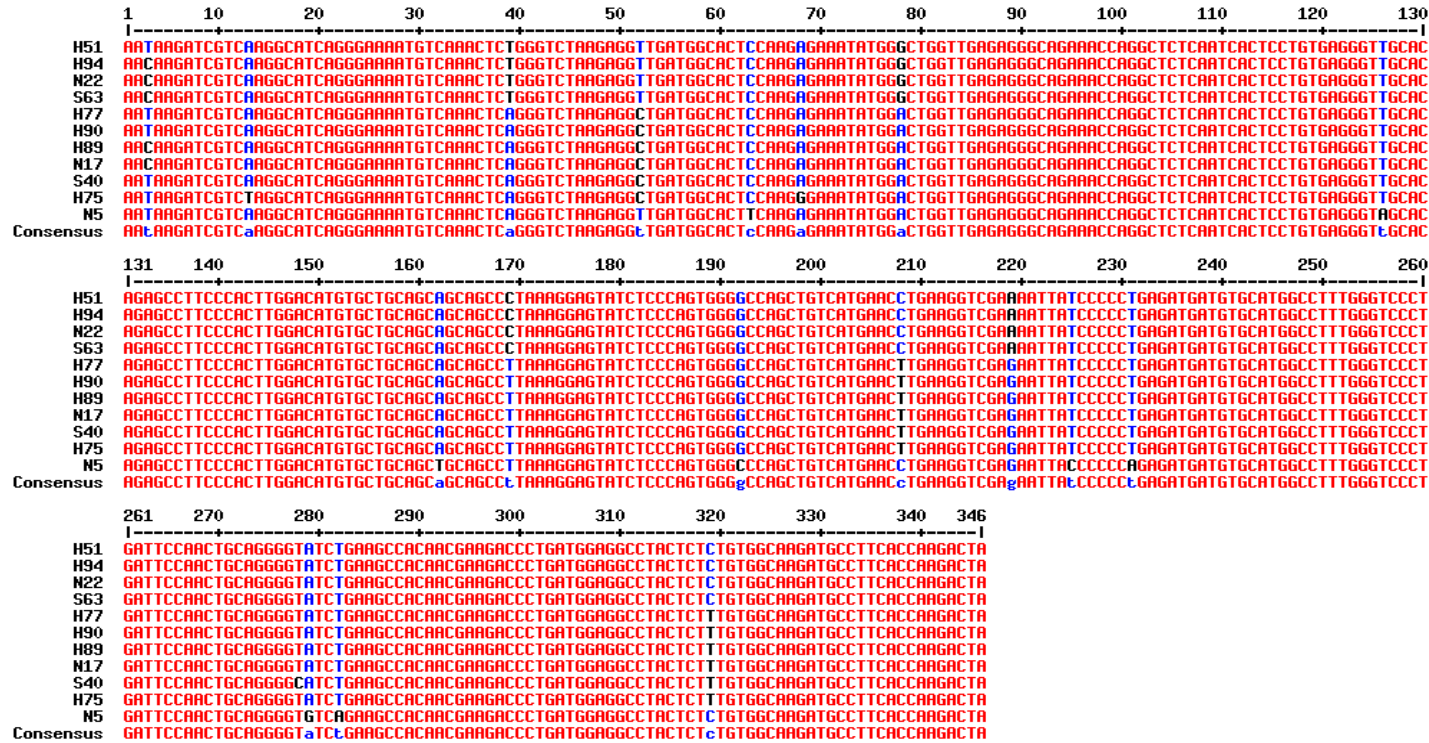
	No. of PCR positive/No. of samples (%)	
	Feral cats	House cats
Sexes		
Male	2/50 (4.0)	3/75 (4.0)
Female	4/51 (7.8)	2/52 (3.9)
Total	6/101 (5.9)	5/127 (3.9)

(SFTSV 2 positive house cats were from feral cats)

Phylogenetic analysis of SFTS virus from cats

from cats

'S' segment



Detection of SFTS virus from **military dogs**



Chuncheon military dog training center

Highly risk group to tick exposure



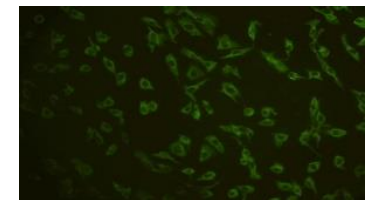
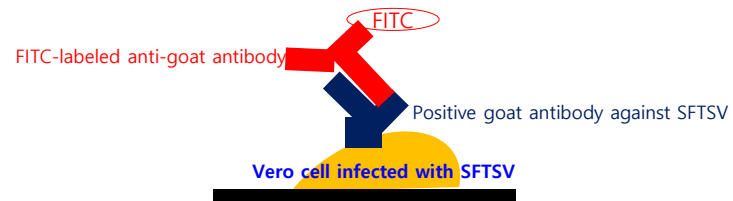
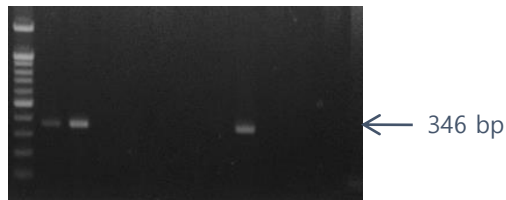
Detection of SFTS virus from dog

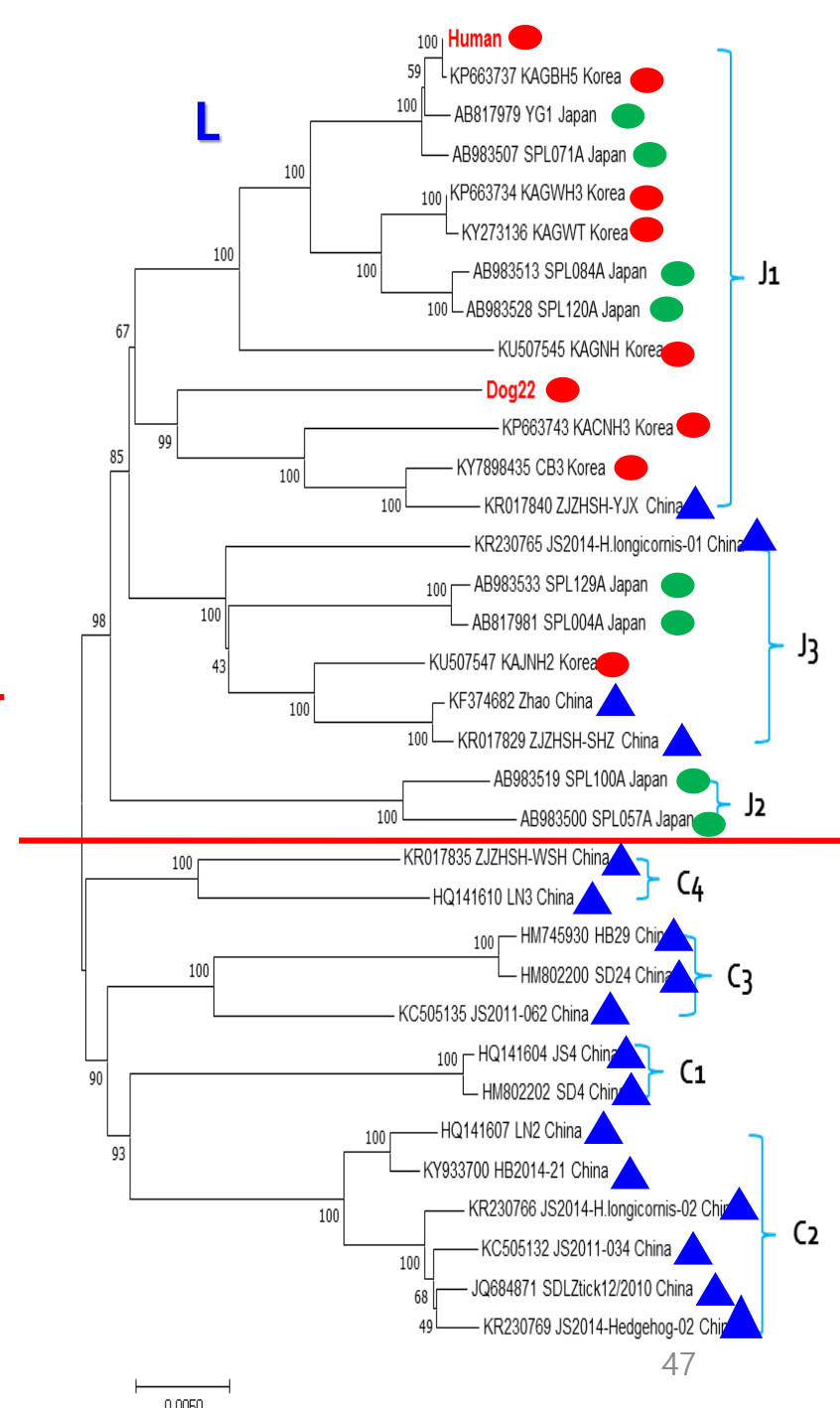
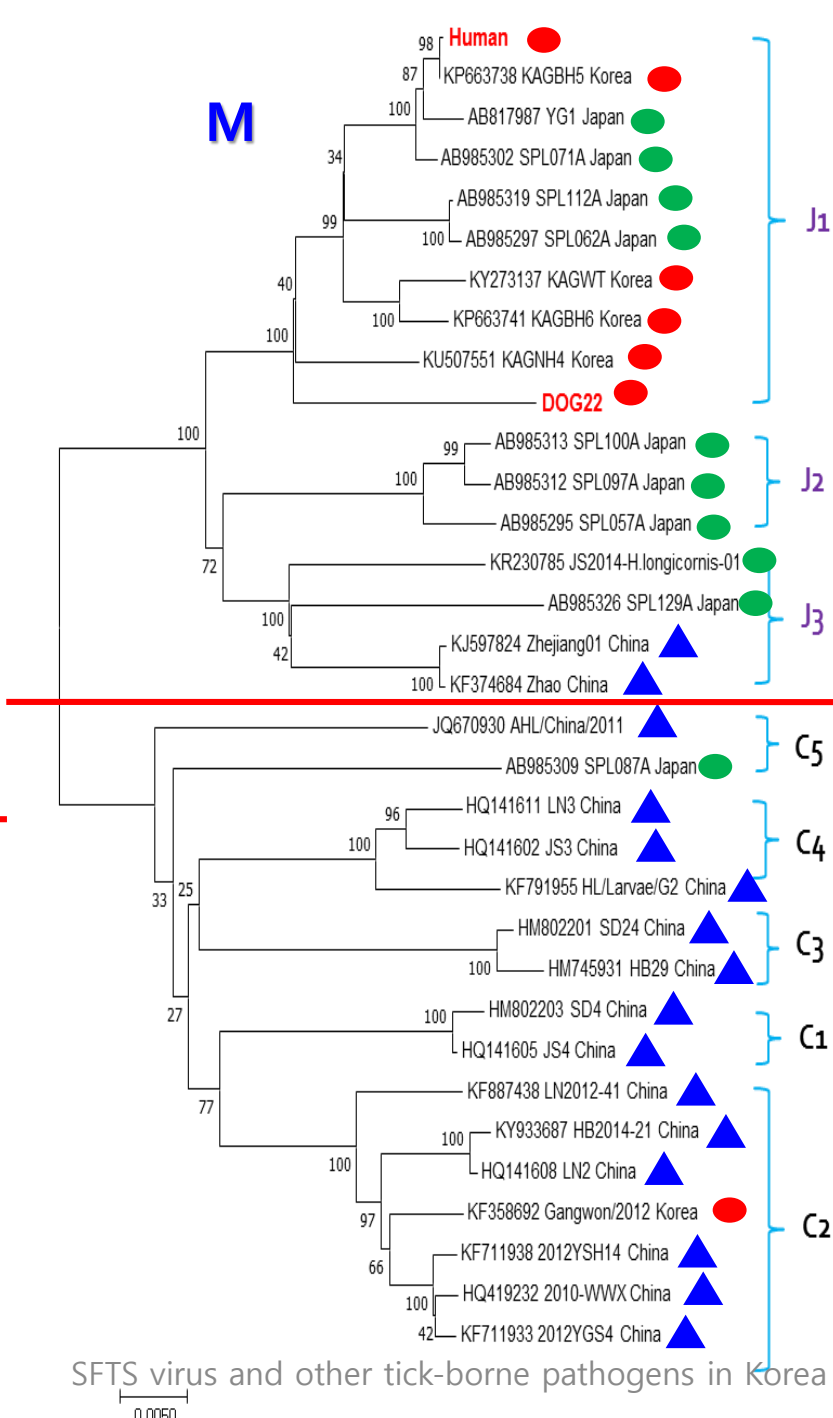
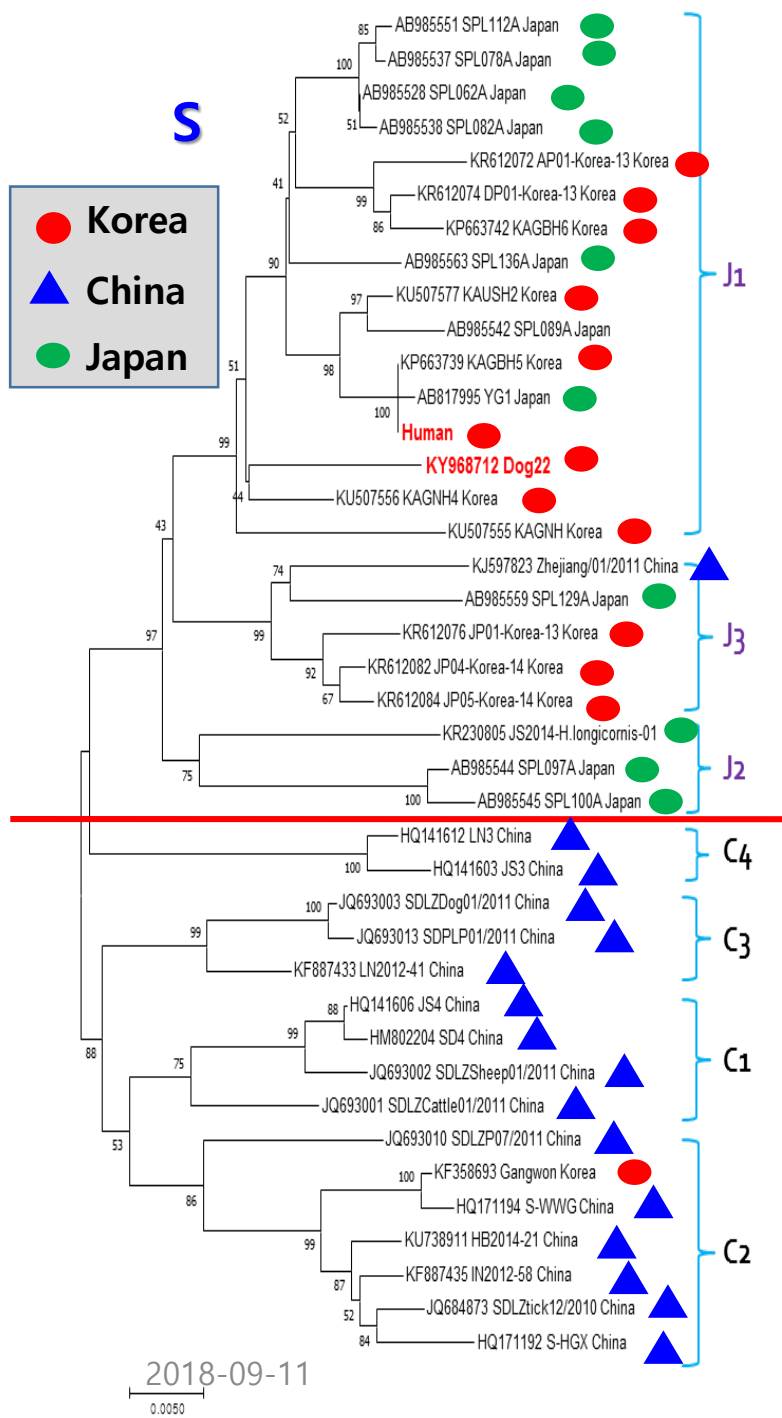
- Antigen test by Nested PCR

Breed	No. of samples	Number of PCR results; No. of positives (%)
German Shepherd	58	2 (3.5%)
Belgian Malinois	42	1 (2.4%)
Labrador Retriever	3	0 (0%)
Total	103	3 (2.9%)

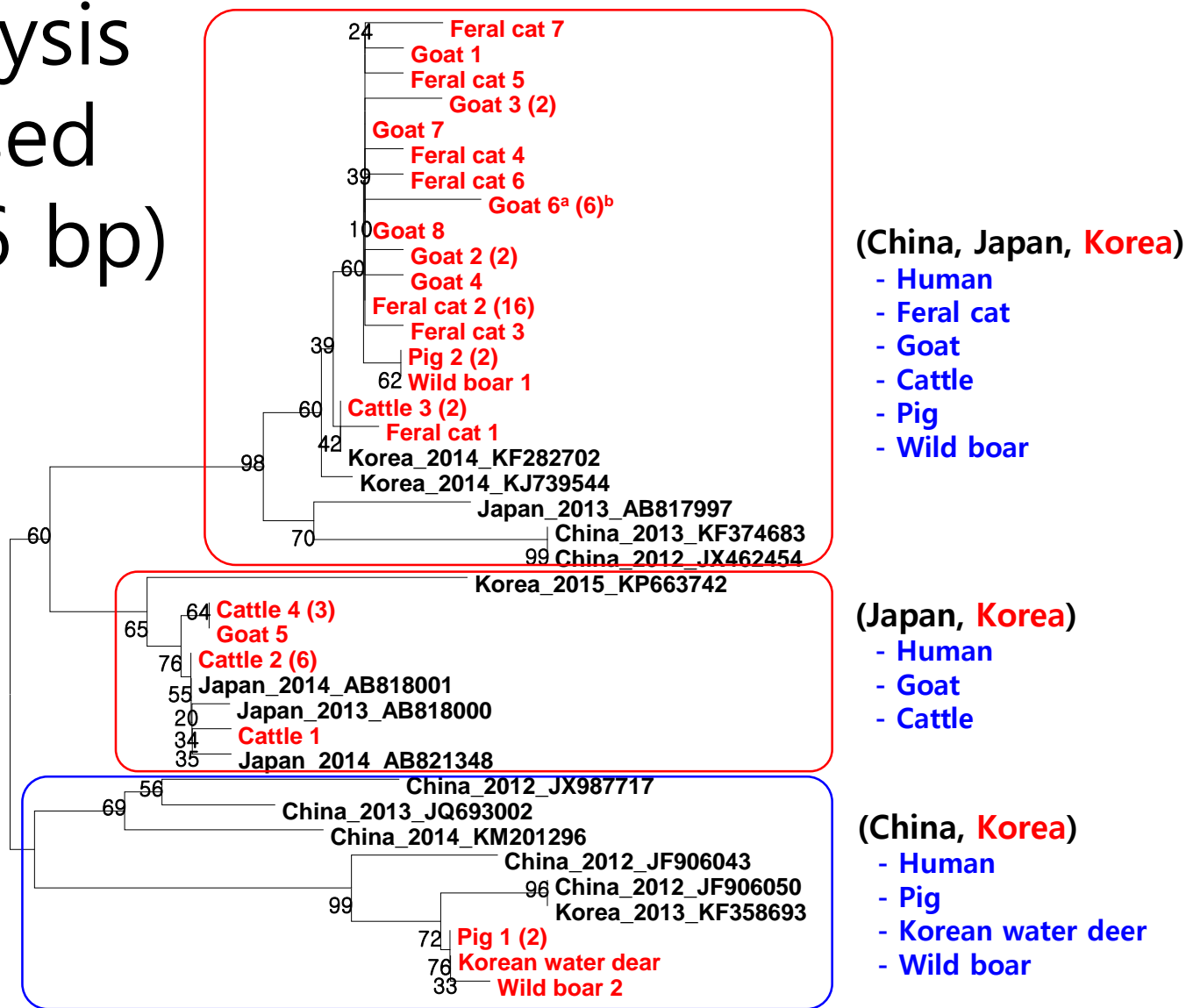
- Antibody test by IFA

Breed	No. of samples	Serum dilution ratio			
		≤1:200	≤1:400	≤1:800	Total
German Shepherd	58	1	2	11	14
Belgian Malinois	42	1	1	6	8
Labrador Retriever	3	0	0	0	0
Total	103	2 (1.9%)	3 (2.9%)	17 (16.5%)	22 (21.4%)

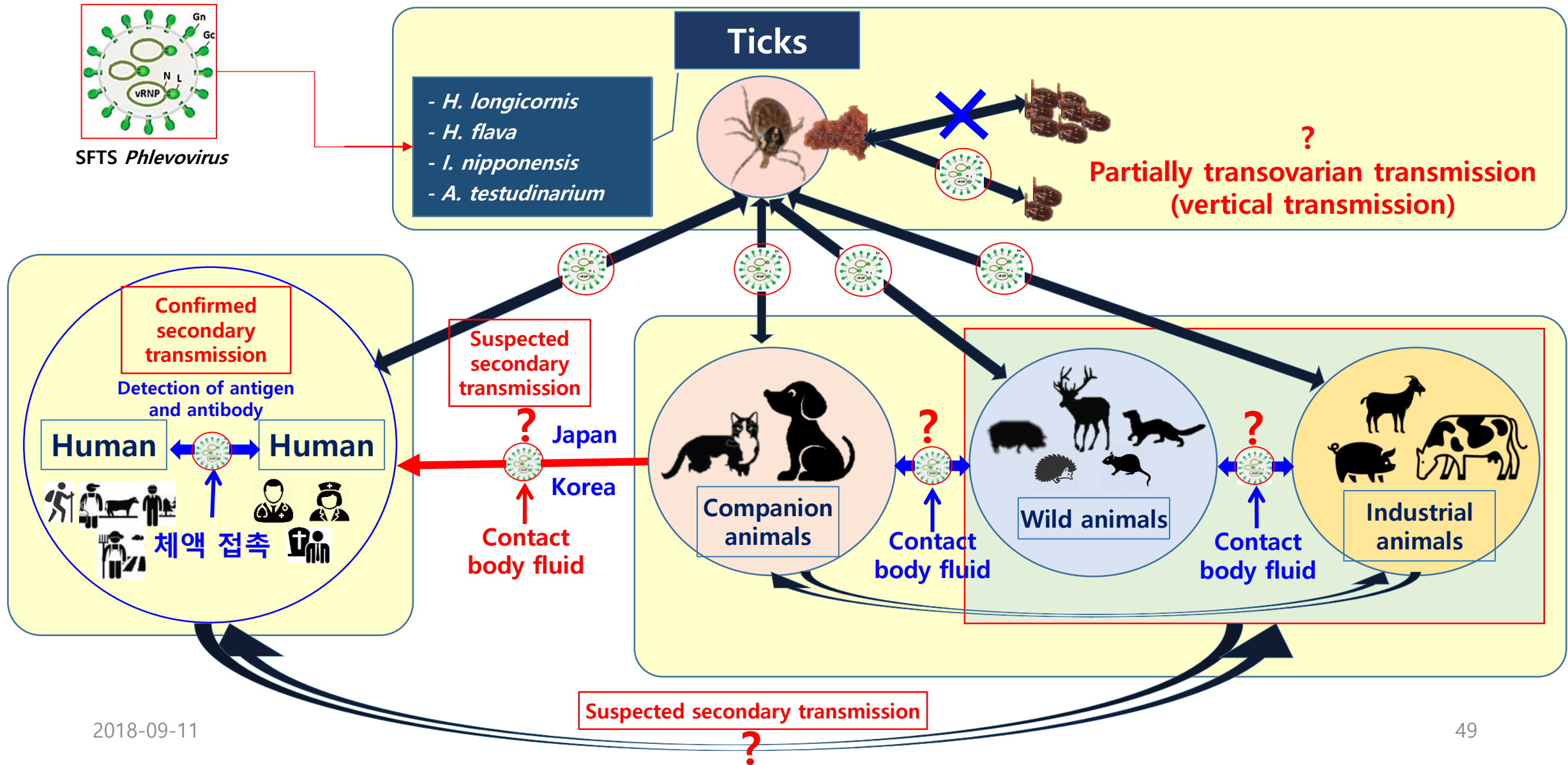




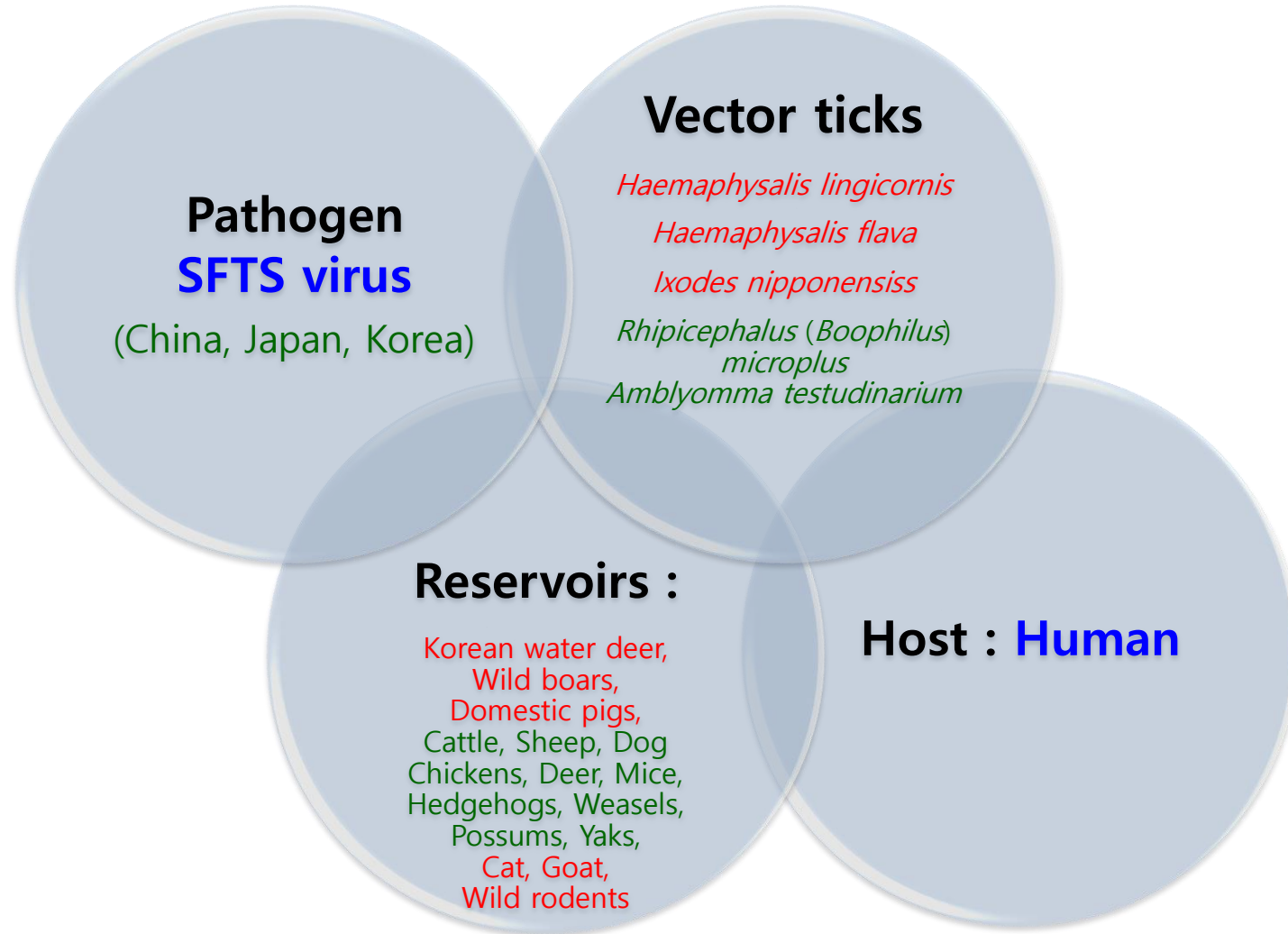
Phylogenetic analysis of SFTS virus based on S segment (346 bp)



Life cycle of *SFTS Phlebovirus* in the nature

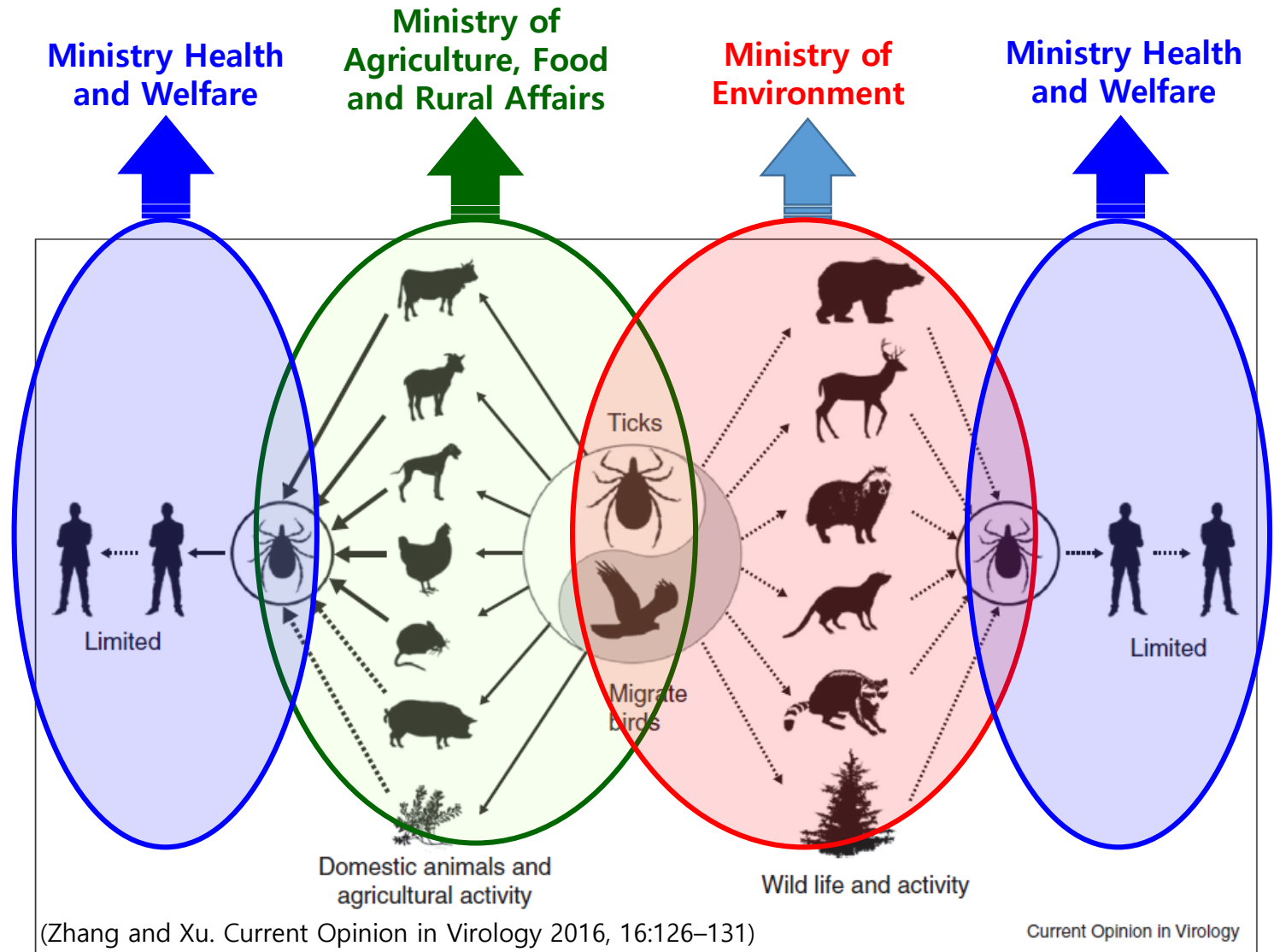


SFTS virus in the world



How to control of tick-borne diseases?

- **One Health**



Other tick-borne pathogens in Korea

- Virus
- Bacteria
- Rickettsia
- Hemoparasites

Recently confirmed tick-borne **virus** in Korea

Pathogens	Vectors	Reservoirs	Hosts	References
SFTS virus	<i>Haemaphysalis lingicornis</i> , <i>H. flava</i> , <i>Ixodes nipponensis</i> , <i>Rhipicephalus (Boophilus) microplus</i> , <i>Amblyomma testudinarium</i>	<i>Korean water deer</i> , <i>Wild boars</i> , <i>Domestic pigs</i> , <i>Cattle</i> , <i>Sheep</i> , <i>Dog</i> , <i>Chickens</i> , <i>Deer</i> , <i>Mice</i> , <i>Hedgehogs</i> , <i>Weasels</i> , <i>Possums</i> , <i>Yaks</i> , <i>Cat</i> , <i>Goat</i> , <i>Wild rodents</i>	Human	Kim et al., 2013
TBE virus	<i>Haemaphysalis longicornis</i> , <i>H. flava</i> , <i>Ixodes nipponensis</i>	<i>Apodemus agrarius</i> , <i>Wild boars</i>		Kim et al., 2008 Ko et al., 2010

Recently confirmed tick-borne **Rickettsia** in Korea

Pathogens	Vectors	Reservoirs	Hosts	References
<i>Anaplasma phagocytophilum</i>	<i>Haemaphysalis longicornis</i> , <i>Ixodes nipponensis</i>	Korean water deer (<i>Hydropotes inermis argyropus</i>), Striped field mice (<i>Apodemus agrarius</i>), Migratory birds	Human, Dog, Cat, Horse, Cattle	Heo et al., 2002 Chae et al., 2003 Park et al., 2003 Kang et al., 2011
<i>A. bovis</i>	<i>Haemaphysalis longicornis</i> , <i>Ixodes turdus</i>	Korean water deer (<i>Hydropotes inermis argyropus</i>)		Kang et al., 2011
<i>A. platys</i>		<i>Apodemus agrarius</i>	Dog	Chae et al., 2003 Kim et al., 2006 Lee et al., 2005
<i>A. centrale</i>	<i>Haemaphysalis longicornis</i>			Oh et al., 2009
<i>Ehrlichia chaffeensis</i>	<i>Haemaphysalis longicornis</i>	<i>Apodemus agrarius</i> , <i>Mus musculus</i>	Human, Cat, Dog	Heo et al., 2002 Chae et al., 2003 Park et al., 2003
<i>E. canis</i>		<i>Apodemus agrarius</i> , <i>Cricetulus triton nester</i>		Chae et al., 2003 Kim et al., 2006
<i>E. ewingii</i>		<i>Apodemus agrarius</i>		Kim et al., 2006
<i>Rickettsia</i> sp.	<i>Haemaphysalis longicornis</i> , <i>H. flava</i> , <i>Ixodes nipponensis</i>			Chae et al., 2008

Recently confirmed tick-borne **Bacteria** in Korea

Pathogens	Vectors	Reservoirs	Hosts	References
<i>Bartonella elizabeth</i>	<i>Haemaphysalis longicornis</i> , <i>H. flava</i> , <i>Ixodes nipponensis</i> , <i>I. turdus</i> , <i>I. persulcatus</i> , <i>Ixodes</i> spp.	<i>Apodemus agrarius</i> , <i>Eothenomys regulus</i> , <i>Crocidura lasiura</i>		Kim et al., 2005 Chae et al., 2008
<i>B. grahamii</i>	<i>Ixodes turdus</i>	<i>Apodemus agrarius</i> Migratory birds		Ko et al., 2014 Kang et al., 2013
<i>B. henselae</i>		Wild rodents		Ko et al., 2014
<i>B. taylorii</i>		Wild rodents		Ko et al., 2014
<i>B. tribocorum</i>		Wild rodents		Ko et al., 2014
<i>B. phoceensis</i>		Wild rodents		Ko et al., 2014
<i>Bartonella</i> sp.			Dog	
<i>Borrelia burgdorferi</i>	<i>Ixodes nipponensis</i>		Human	Chae et al., 2008
<i>B. turdi</i>	<i>Ixodes nipponensis</i> <i>Ixodes turdus</i>			Kang et al., 2013
<i>Borrelia</i> sp.	<i>Ixodes nipponensis</i> <i>Ixodes turdus</i>	Migratory birds		Kang et al., 2013

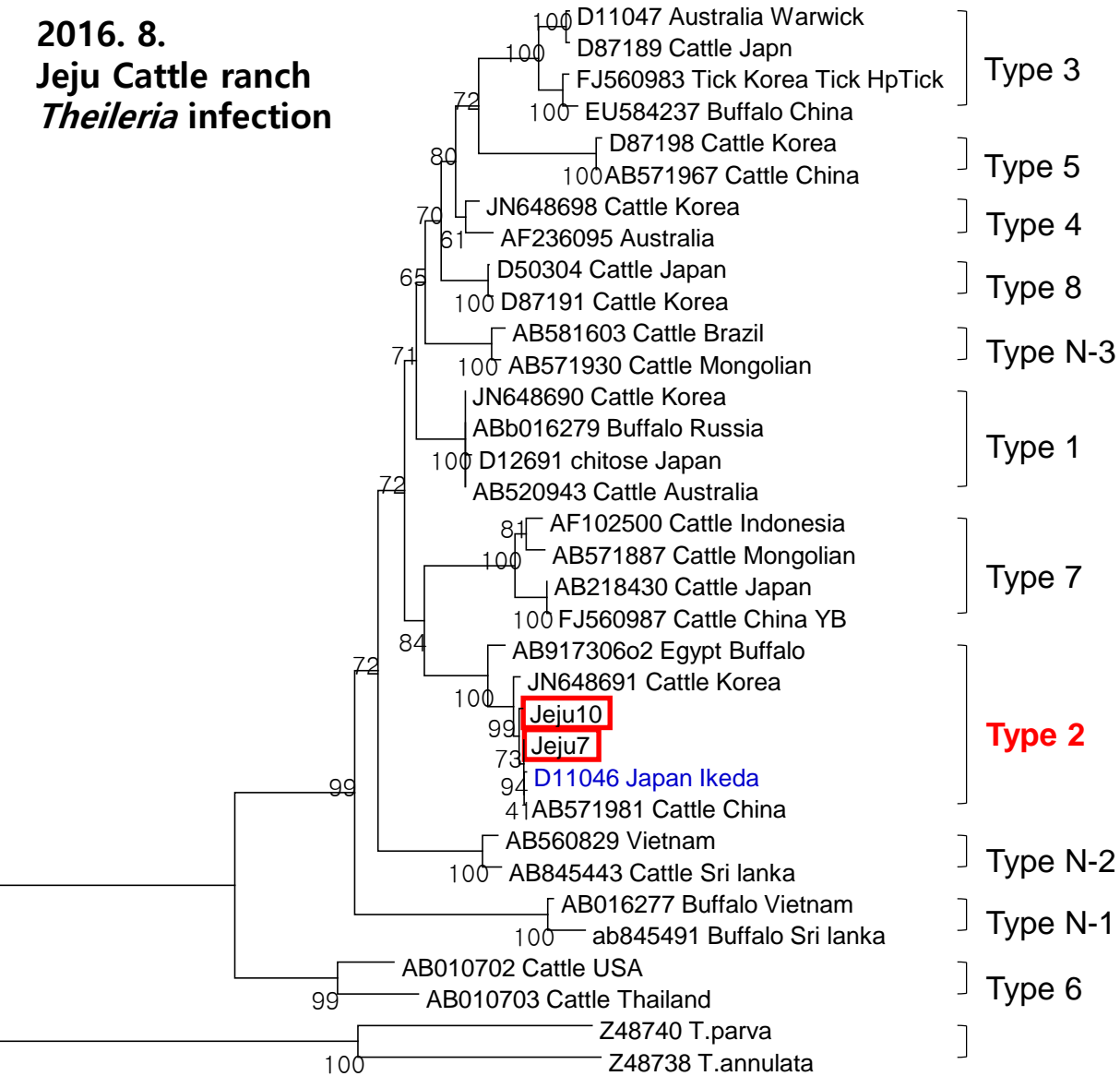
Recently confirmed tick-borne **hemo-parasite** in Korea

Pathogens	Vectors	Reservoirs	Hosts	References
<i>Babesia gibsoni</i>	<i>Haemaphysalis longicornis</i>		Dog	Lee et al., 2009
<i>Theileria sergenti/buffeli/orientalis</i>	<i>Haemaphysalis longicornis</i>		Cattle	Chae et al., 1998 Kim et al., 2017 Park et al., 2017
<i>Theileria luwenshuni</i>		Korean water deer		Seong et al., 2015 Lee et al., 2016
<i>Theileria ovis</i>				Lee et al., 2016

Detection of tick-borne pathogen from cattle in Jeju island (2016. 8)

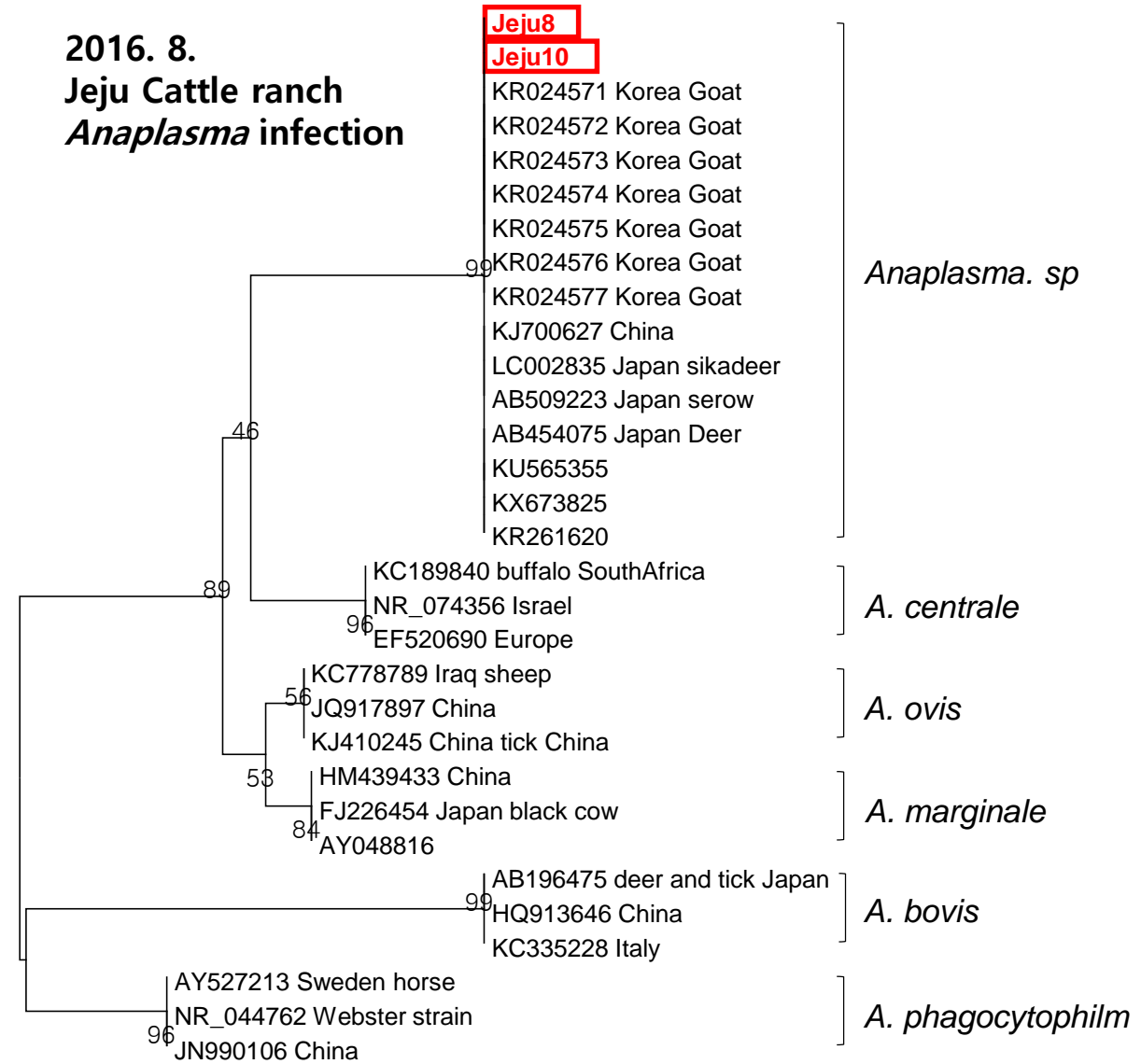
Sample No.	<i>Theileria</i>	<i>Anaplasma</i>	<i>Ehrlichia</i>
1	+	+	-
2	+	+	-
3	+	+	-
4	+	+	-
5	+	+	-
6	+	+	-
7	+	+	-
8	+	+	-
9	+	+	-
10	+	+	-
11	+	+	-
12	+	+	-
13	+	+	-
14	+	+	-
15	+	+	-
16	+	+	-

2016. 8.
Jeju Cattle ranch
Theileria infection



0.1 2018-09-11

2016. 8.
Jeju Cattle ranch
Anaplasma infection



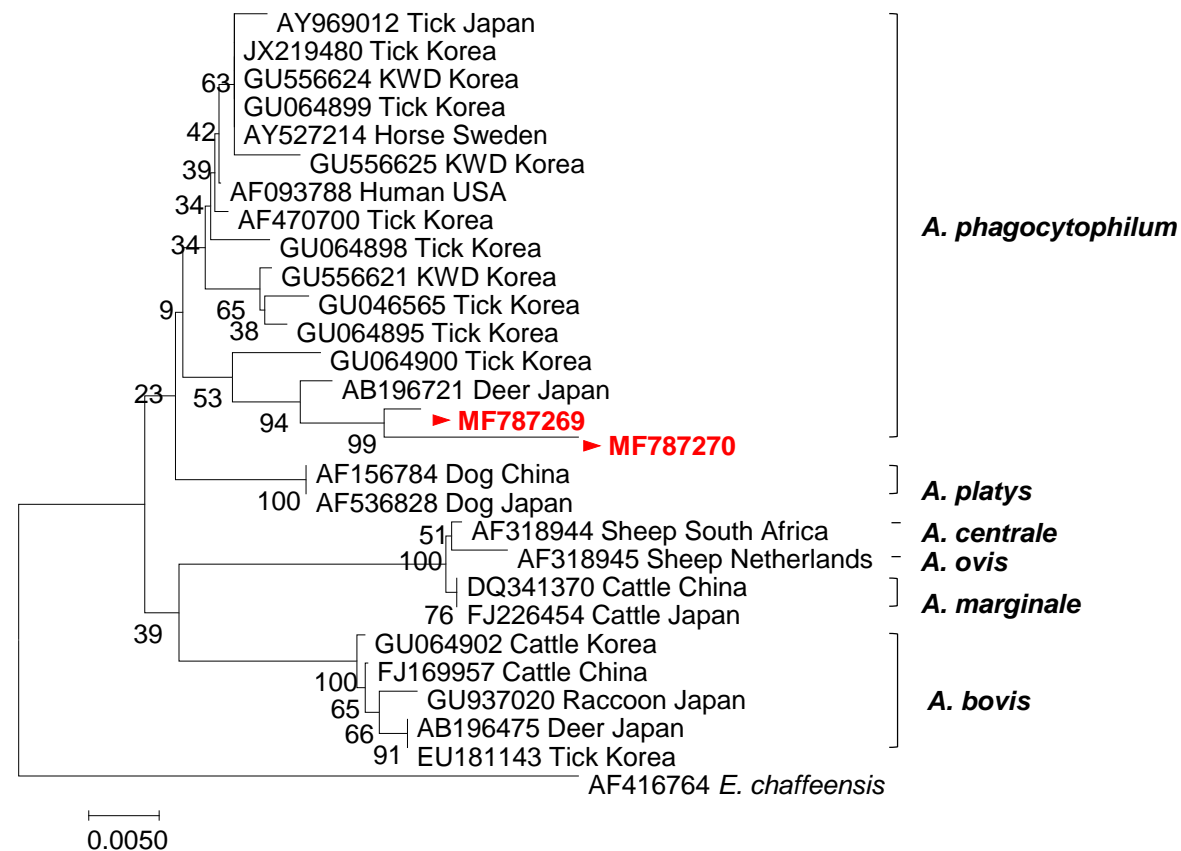
0.005

Molecular detection of tick-borne pathogens in Holstein cattle in the Republic of Korea

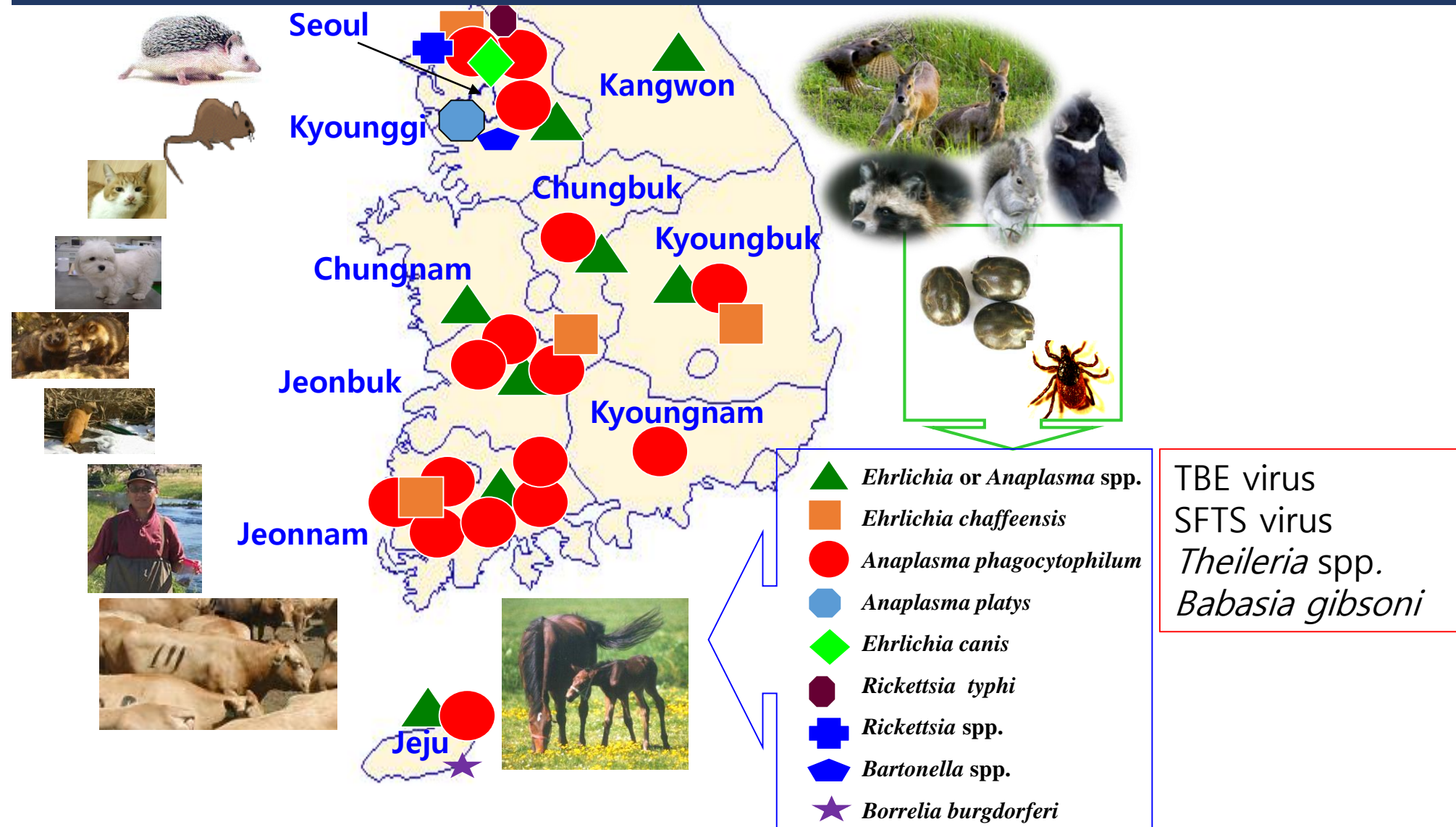
Han DG, Ryu JH, Chae JB, Chae JS, Park J, Yu DH, Park BK, Kim HC, Choi KS

Summary of *T. orientalis* and *A. phagocytophilum* infection by PCR

Sample ID	Age (Months)	Tick-borne pathogens				
		<i>T. orientalis</i>				<i>A. phagocytophilum</i>
		MPSP	Chitose	Ikeda	Chitose + Ikeda	
53	25	-	-	-	-	-
55	23	+	-	-	+	+
56	21	-	-	-	-	-
57	19	-	-	-	-	-
59	17	+	-	+	-	-
63	12	+	-	-	+	-
64	12	-	-	-	-	-
65	11	-	-	-	-	-
210	54	+	-	-	+	+
510	15	+	-	+	-	-
511	15	+	-	+	-	-
601	13	+	+	-	-	-



Detection of tick-borne pathogens in Korea



Summary of study

- We confirmed many kinds of tick-borne pathogens from ticks and animals in Korea.
- SFTS virus were detected from several tick species, domestic, companion and wild animals, also human.
- One of the study is the first reported about detection of *A. phagocytophilum* and *A. bovis* in Korean water deer.
- Four genotypes of *A. phagocytophilum* and 1 genotype of *A. bovis* are new genetic variants from Korean water deer.
- *Theileria* spp. infection is the prevalent in the livestock and wild animals of the Korea.
- *T. orientalis* infection rate was relatively high. The frequency of the transmission of the pathogenic genotype (Type 2) seems to have increased, and thereby, might pose a significant risk to cattle health.
- Wild animals may act as reservoir of SFTS virus, *Anaplasma* and other tick-borne pathogens.
- We need more study about transmission among animals and its confirmation.
- Future studies need for culture isolation and characterization of pathogens.
- Further studies should be emphasized on effective monitoring and prevention programs through epidemiological surveys.

Funding Resources

- Environmental Health Research Division, National Institute of Environmental Research
- National Institute of Biological Resources
- National Park Research Institute, Korea National Park Service
- Agriculture Science and Technology Development, Rural Development Administration
- Animal and Plant Quarantine Agency
- National Research Foundation of Korea
- BK21 PLUS Program for Creative Veterinary Science Research, Seoul National University

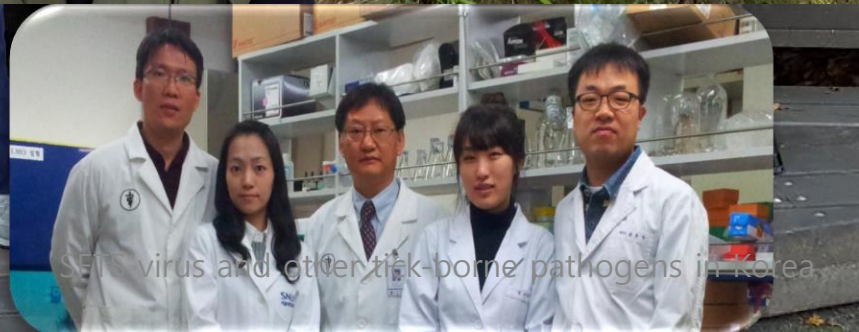
Thank you for your attention



금강모시가 살아 숨쉬는 곳 - 구천동 자연 관찰로



2018-09-11



SFTS virus and other tick-borne pathogens in Korea



References

Detection of Severe Fever with Thrombocytopenia Syndrome Virus from Wild Animals and Ixodidae Ticks in the Republic of Korea

Sung-Suck Oh^{1,2}, Jeong-Byoung Chae¹, Jun-Gu Kang¹, Heung-Chul Kim³, Sung-Tae Chong³, Jeong-Hwa Shin⁴, Moon-Suk Hur⁴, Jae-Hwa Suh⁴, Myoung-Don Oh⁵, Soo-Myoung Jeong⁶, Nam-Shik Shin⁷, Kyoung-Seong Choi⁸, and Joon-Seok Chae¹

Abstract

Severe fever with thrombocytopenia syndrome (SFTS) is caused by SFTS virus (SFTSV), a novel bunyavirus reported to be endemic to central-northeastern China, southern Japan, and the Republic of Korea (ROK). To investigate SFTSV infections, we collected serum samples and ticks from wild animals. Using serum samples and ticks, SFTSV-specific genes were amplified by one-step RT-PCR and nested PCR and sequenced. Indirect immunofluorescence assay (IFA) was performed to analyze virus-specific antibody levels in wild animals. Serum samples were collected from a total of 91 animals: 21 Korean water deer (KWD), 3 Siberian roe deer, 5 goral, 7 raccoon dogs, 54 wild boars (WBs), and 1 carrion crow. The SFTSV infection rate in wild animals was 3.30% (3 of 91 animals: 1 KWD and 2 WBs). The seropositive rate was 6.59% (6 of 91 animals: 5 KWD and 1 WB). A total of 891 ticks (3 species) were collected from 65 wild animals (9 species). Of the attached tick species, *Haemaphysalis longicornis* (74.86%) was the most abundant, followed by *Haemaphysalis flava* (20.20%) and *Ixodes nipponensis* (4.94%). The average minimum infection rate (MIR) of SFTSV in ticks was 4.98%. The MIRs of *H. longicornis*, *H. flava*, and *I. nipponensis* were 4.51%, 2.22%, and 22.73%, respectively. The MIRs of larvae, nymphs, and adult ticks were 0.68%, 6.88%, and 5.53%, respectively. In addition, the MIRs of fed and unfed ticks were 4.67% and 4.96%, respectively. We detected a low SFTSV infection rate in wild animals, no differences in SFTSV infection rate with respect to bloodsucking in ticks, and SFTSV infection for all developmental stages of ticks. This is the first report describing the detection of SFTSV in wild animals in the ROK.

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SFTS virus and other tick-borne pathogens in Korea



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Short communication

Molecular detection of severe fever with thrombocytopenia syndrome virus (SFTSV) in feral cats from Seoul, Korea



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ABSTRACT

This study tested serum samples of feral cats from a highly urbanized habitat, Seoul, Korea to determine the infection to severe fever with thrombocytopenia syndrome virus (SFTSV). From 126 samples tested, SFTSV was detected by RT-PCR in 22 (17.5%) cats from various sites of Seoul. Sequences identified from this study were grouped with clusters from China and Japan. Our result provides data that SFTSV may have been circulating in settings that were suspected to have relatively low risk, such as highly urbanized habitats. Thus it warrants further study to investigate the ecology of SFTSV in urban-dwelling animals including ticks, human and other potential host species.

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Molecular Detection of Severe Fever with Thrombocytopenia Syndrome Virus in Korean Domesticated Pigs

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Yoon-Kyoung Cho¹, and Joon-Seok Chae¹

Abstract

Background: Severe fever with thrombocytopenia syndrome virus (SFTSV) is an emerging tick-borne virus in China, Japan, and Korea. Although the incidence of SFTS has been increasing annually since the first SFTSV case was reported in Korea, the prevalence of SFTSV in Korean livestock has not been conducted. The purpose of this study was to investigate the prevalence of SFTSV in domesticated pigs (*Sus scrofa domestica*) throughout Korea.

Methods: A total of 240 sera were collected from 30 domesticated pigs in eight provinces. To detect SFTSV, one-step reverse transcription RT nested PCR was conducted using SFTSV genome-specific primer sets. The amplified PCR products were cloned and then sequenced.

Results: Four of 240 (1.7%) pig sera tested positive for SFTSV through one-step RT nested PCR. Two of the four obtained sequences were included in the Japanese clade, whereas the other two sequences were related to the Chinese clade based on phylogenetic analysis. Moreover, the obtained SFTSV sequences from this study were distinct from human SFTSV strains.

Conclusions: This study is the first to attempt the identification of SFTSV sequences from domesticated pigs and the first molecular detection of SFTSV in Korean livestock. Our findings indicate that a new subclade of SFTSV—different from that in humans—may be present in domesticated pigs; the surveillance of SFTSV in livestock is required to better understand the life cycle of SFTSV.

Keywords: severe fever with thrombocytopenia syndrome virus, Bunyaviridae, pig, PCR, Korea



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Original article

Prevalence of severe fever with thrombocytopenia syndrome virus in black goats (*Capra hircus coreanae*) in the Republic of Korea

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ABSTRACT

Severe fever with thrombocytopenia syndrome virus (SFTSV) is an emerging tick-borne pathogen in China, Japan, and the Republic of Korea (ROK). The aim of this study was to investigate the prevalence of SFTSV antigens and anti-SFTSV antibodies in black goats (*Capra hircus coreanae*) throughout the ROK. Sera were collected from 737 black goats in nine provinces in the ROK. Eighteen of 737 (2.4%) goat sera were positive for SFTSV on one-step reverse transcription nested polymerase chain reaction. The amplified 346-bp S segments of SFTSV sequences were classified into three genotypes (BG1, BG2, and BG3), and were included in the Japanese clade rather than the Chinese clade, based on phylogenetic analysis. Forty-three of 624 (6.9%) serum samples were seropositive for anti-SFTSV antibodies on enzyme-linked immunosorbent assay analysis. This study is the first to examine the molecular prevalence of SFTSV in goats and the first to perform serological detection of anti-SFTSV antibodies in livestock in the ROK. Moreover, the results indicate that SFTSV is widely distributed in goats and that additional monitoring for SFTSV is needed in livestock in the ROK.

Molecular Detection of *Anaplasma*, *Bartonella*, and *Borrelia theileri* in Raccoon Dogs (*Nyctereutes procyonoides*) in Korea

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Abstract. Anaplasmosis, cat-scratch disease, and Lyme disease are emerging vector-borne infectious diseases in Korea. Although the prevalence of vector-borne pathogens (VBPs) in domestic animals and vector arthropods has been documented, there is limited information on the presence of VBPs in wild animals. The raccoon dog (*Nyctereutes procyonoides*), a wild canid found in East Asia and Europe, represents a potential wildlife reservoir for zoonotic diseases. To investigate the prevalence of VBPs in raccoon dogs, 142 carcasses and 51 blood samples from captured raccoon dogs were collected from 2003 to 2010 and from 2008 to 2009, respectively, in Korea. In addition, 105 *Haemaphysalis flava* (14 larvae, 43 nymphs, 32 males, and 16 females) and nine *Haemaphysalis longicornis* (all female) were collected from three raccoon dogs. Samples of the spleen and blood were tested for the presence of VBPs by using nested polymerase chain reaction. Among the samples collected from 193 raccoon dogs and 114 ticks, two samples were positive for *Anaplasma phagocytophilum*, four for *Anaplasma bovis*, two for *Borrelia theileri*, and two for *Bartonella henselae*. To the best of our knowledge, this study is the largest survey of raccoon dogs aimed at the analysis of VBPs in this species. Moreover, the present study represents the first identification of *A. phagocytophilum*, *B. henselae*, and *B. theileri* in raccoon dogs in their native habitat (East Asia).

ORIGINAL ARTICLE

Identification of Tick Species Collected from Wild Boars and Habitats of Wild Boars and Domestic Pigs in the Republic of Korea

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Abstract: Tick is one of the most important arthropods in the transmission of vector-borne diseases. In this study, we investigated the abundance and species of ticks associated with swine and their habitats to assess the risk of spread of tick-borne diseases in host species, such as wild boars. Ticks were collected from 24 grazing or traditionally reared domestic pig farms and 8 habitats of wild boars in 8 provinces and 1 city in the Republic of Korea, by using the dragging and flagging methods. Ticks were also collected directly from 49 wild boars by using fine forceps. A total of 9,846 hard ticks were collected, including 4,977 *Haemaphysalis longicornis*, 4,313 *Haemaphysalis flava*, 508 *Ixodes nipponensis*, 1 *Ixodes turdus*, and 47 *Amblyomma testudinarium*. A total of 240 hard ticks were collected from 49 wild boars, including 109 *H. flava*, 84 *H. longicornis*, and 47 *A. testudinarium*. A total of 578 hard ticks were collected from areas around domestic pig farms. Only 2 hard tick species, 546 *H. longicornis* and 32 *H. flava*, were collected from these areas. A total of 9,028 hard ticks were collected from wild boars of 8 habitats, including 4,347 *H. longicornis*, 4,172 *H. flava*, 508 *I. nipponensis*, and 1 *I. turdus*. *A. testudinarium* was collected only from wild boars, and *I. nipponensis* and *I. turdus* were collected only from the habitats of wild boars.



Key words: *Haemaphysalis longicornis*, *Haemaphysalis flava*, *Ixodes nipponensis*, *Ixodes turdus*, *Amblyomma testudinarium*, tick, wild boar, pig

Isolation of Tick-Borne Encephalitis Viruses from Wild Rodents, South Korea

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Abstract

To determine whether the tick-borne encephalitis virus (TBEV) is present in vector ticks and mammalian hosts in Korea, we examined two tick species, *Haemaphysalis longicornis* ($n = 548$) and *Ixodes nipponensis* ($n = 87$), and the lungs or spleens of rodents *Apodemus agrarius* ($n = 24$) and wild boars ($n = 16$). Tick-borne encephalitis virus was detected in samples by reverse transcriptase (RT)-nested polymerase chain reaction (PCR), after which TBEV-positive samples were inoculated into BHK-21 cells and suckling mice. Tick-borne encephalitis virus genes were detected in 4 of 38 tick pools and 5 of 24 wild rodents. Suckling mice inoculated intracerebrally with TBEV-positive rodent samples showed signs of encephalitis at six days post-inoculation. The isolation of TBEV was confirmed by inoculating samples obtained from the brains of sick mice in cell culture. Phylogenetic analysis showed that the E genes of the TBEV isolates were clustered with the Western subtype (98% identity). This study suggests the possible occurrence of tick-borne encephalitis in Korea.

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Original Article

Prevalence of tick-borne encephalitis virus in ticks from southern Korea

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The prevalence of tick-borne encephalitis virus (TBEV) in southern Korea was determined by collecting ticks using tick drags. A total of 4,077 of 6,788 ticks collected were pooled (649 pools) according to collection site, species, and developmental stage and assayed for TBEV. The TBEV protein E and NS5 gene fragments were detected using RT-nested PCR in six pools of nymphs collected from Jeju Island (2,491 ticks). The minimum field detection rates for TBEV were 0.17% and 0.14% for *Haemaphysalis longicornis* and *Haemaphysalis flava* nymphs, respectively. The 252 bp NS5 and 477 bp protein E gene amplicons were sequenced. Phylogenetic analysis showed that the NS5 and protein E genes of the Jeju strain were clustered with Western subtype (98.0% and 99.4% identity, respectively). The Western subtype of TBEV is endemic in Korea, including Jeju Island. The study of vector and zoonotic host susceptibility to TBEV is required to better understand its potential impact on public health.

Keywords: *Haemaphysalis flava*, *Haemaphysalis longicornis*, Korea, tick, tick-borne encephalitis virus

Lyme Patient in Korea

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Serologically Diagnosed Lyme Disease Manifesting Erythema Migrans in Korea

Lyme disease is a vector-borne infection, primarily transmitted by *Ixodes* ticks, and caused by *Borrelia burgdorferi*. It has a wide distribution in the northern hemisphere. In Korea, however, only one human case has been reported, although *B. burgdorferi* was isolated from the vector tick *I. persulcatus* in the region. A 60-year-old male and a 45-year-old female developed the clinical sign of erythema migrans. Each patients were bitten by a tick four weeks and five weeks, respectively, before entering the hospital. On serologic examination, significantly increased IgM and IgG antibody titers to *B. burgdorferi* were observed in consecutive tests performed at an interval of two weeks. They responded well to treatment with tetracycline.

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Serological and molecular prevalence of canine vector-borne diseases (CVBDs) in Korea.

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Abstract

BACKGROUND: Previous surveys in dogs from Korea indicated that dogs are exposed to a variety of vector-borne pathogens, but perception for a nation-wide canine vector-borne disease (CVBD) occurrence has been missing. We report here results of both serological and molecular prevalence studies for major CVBDs of dogs from all over the South Korean Peninsula except for Jeju Island.

RESULTS: Serological survey of 532 outdoor dogs revealed the highest prevalence for *Dirofilaria immitis* (25.2%), followed by *Anaplasma phagocytophilum* (15.6%), *Ehrlichia canis* (4.7%) whereas *Borrelia burgdorferi* showed the lowest prevalence (1.1%). The number of serologically positive dogs for any of the four pathogens was 216 (40.6%). Concurrent real-time PCR assay of 440 dogs in the study indicated that DNA of "Candidatus *M. haematoparvum*", *Mycoplasma haemocanis*, *Babesia gibsoni*, *A. phagocytophilum*, and *Hepatozoon canis* was identified in 190 (43.2%), 168 (38.2%), 23 (5.2%), 10 (2.3%) and 1 (0.2%) dogs, respectively. DNA of *Bartonella* spp., *Ehrlichia* spp., *Leishmania* spp., *Rickettsia* spp. and *Neorickettsia risticii* was not identified. Analysis of questionnaires collected from owners of 440 dogs showed that the number of dogs with heartworm preventive medication was 348 (79.1%) among which dogs still positive to *D. immitis* infection were 60 (17.2%), probably due to the mean months of heartworm preventive medication being only 6.5. The high prevalence rates of both "Ca. *M. haematoparvum*" and *Mycoplasma haemocanis* in dogs from Korea indicate that these organisms may be transmitted by vectors other than *Rhipicephalus sanguineus* because this tick species has rarely been found in Korea. This is the first nationwide survey for canine haemotropic mycoplasma infections in Korea.

CONCLUSIONS: This study showed that the risk of exposure to major vector-borne diseases in dogs is quite high throughout all areas of South Korean Peninsula. Since achieving full elimination of many pathogens causing CVBDs from infected animals is often impossible even when they are clinically cured, dogs once exposed to CVBDs can remain as lifetime reservoirs of disease for both other animals and humans in the close vicinity, and should therefore be treated with preventative medications to minimise the risk of pathogen transmission by the competent vectors.



Molecular Detection of *Anaplasma*, *Bartonella*, and *Borrelia* Species in Ticks Collected from Migratory Birds from Hong-do Island, Republic of Korea

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Ehrlichia and *Borrelia* spp. Infection in German Shepherd Dogs in Korea

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(Accepted: April 16, 2011)

Abstract : The presence of the tick-borne pathogens *Ehrlichia* and *Borrelia* in German Shepherd dogs in Korea was determined by enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR). A total of 291 dogs were randomly selected from five Korean provinces from October 2005 through September 2006. The seroprevalence of antibodies to canine *Ehrlichia* and *Borrelia* agents detected by ELISA (Snap[®] 3Dx[®] Test, IDEXX Laboratories) was 7.56% (22 dogs) and 1.72% (5 dogs) respectively, throughout the country. Positive antibodies against both pathogens were detected in two dogs (0.69%). The provincial distribution of seroprevalence against *Ehrlichia* was 1.28% (1 of 78) in Gyeonggi-do, 12.64% (11 of 87) in Gangwon-do, 9.76% (4 of 41) in Chungcheong-do, 8.93% (5 of 56) in Gyeongsang-do, and 3.45% (1 of 29) in Jeolla-do. According to PCR analysis, *Ehrlichia chaffeensis* target DNA was amplified in 3.09% (9 of 291 dogs) of blood samples, 2.41% (7 of 291) from Gangwon-do and 0.69% (2 of 291) from Chungcheong-do. The oligonucleotide sequences (SNU-EC3 and SNU-EC5) from the PCR fragment examined in Korea were closely related to *E. chaffeensis* isolated from the tick *Haemaphysalis longicornis*, in China and the state of Arkansas in the US. Based on these results, the presence of *E. chaffeensis* infection was identified in German Shepherds being bred in Korea. These results bring to light the importance of paying close attention to tick-borne infections such as Lyme disease during clinical diagnosis. This infectious disease should be included as a differential diagnosis for patients who participate in outdoor activity from spring to fall or who have thrombocytopenia or leucopenia.

Abstract

Bird migration is a recurring annual and seasonal event undertaken by more than 100 species of birds in the southeast Asian and northeast Palearctic regions that pass through or remain for short periods from April to May and September to November at Hong-do Island, Republic of Korea (ROK). A total of 212 ticks (40 *Haemaphysalis flava*, 12 *H. longicornis*, 146 *Ixodes turdus*, 13 *I. nipponensis*, and 1 *I. ornithophila*) were collected from 65/2,161 (3.0%) migratory birds consisting of 21 species that were captured from January, 2008, through December, 2009, as part of the Migratory Birds Center, Hong-do bird banding program for studying bird migration patterns. Adult ticks were assayed individually while larvae and nymphs were pooled (1–22 and 1–6 ticks per pool, respectively) into 31 and 65 pools, respectively. Ticks were assayed for zoonotic pathogens by PCR using 16S rRNA, heat shock protein (*groEL*), and internal transcribed spacer (ITS) gene primers to amplify genera specific for *Anaplasma*, *Bartonella*, and *Borrelia* PCR amplicons. Using the 16S rRNA-based nested PCR, *A. phagocytophilum* ($n=1$) was detected in *I. nipponensis* collected from *Zoothera sibirica* and *A. bovis* ($n=1$) was detected in *I. turdus* collected from *Emberiza chrysophrys*. *Borrelia turdi* 16S rRNA genes ($n=3$) were detected in *I. turdus* and *I. nipponensis* collected from *Turdus pallidus* and *Zoothera aurea*. *Borrelia* spp. 16S rRNA genes ($n=4$) were detected in *Ixodes* ticks collected from *Emberiza tristrami*, *T. pallidus*, and *Z. aurea*. The *Bartonella grahamii* ITS gene ($n=1$) was detected by nested PCR assay in *I. turdus* collected from *Z. aurea*. These results provide insight into the potential role of migratory birds in the dispersal of ticks and associated tick-borne pathogens throughout their ranges in Asia.

Original Article

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Prevalence of *Anaplasma*, *Bartonella* and *Borrelia* Species in *Haemaphysalis longicornis* collected from goats in North Korea

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North Korea is located on the northern part of the Korean Peninsula in East Asia. While tick-borne pathogens of medical and veterinary importance have been reported from China and South Korea, they have not been reported from North Korea. To screen for zoonotic tick-borne pathogens in North Korea, ticks were collected from domestic goats. A total of 292 (27 nymph, 26 male, 239 female) *Haemaphysalis (H.) longicornis* were collected and assayed individually for selected tick-borne pathogens. A total of 77 (26.4%) were positive for *Anaplasma bovis*, followed by *Bartonella (B.) grahamii* (15, 5.1%), *Anaplasma phagocytophilum* (12, 4.1%), *Bartonella henselae* (10, 3.4%), and *Borrelia* spp. (3, 1.0%) based on 16S ribosomal RNA and ITS species-specific nested polymerase chain reaction. Using the *groEL*-based nested PCR, a total of 6 and 1 *H. longicornis* were positive for *B. grahamii* and *B. henselae*, respectively. All products were sequenced and demonstrated 100% identity and homology with previously reported sequences from other countries in GenBank. This is the first report of the detection of tick-borne pathogens in the North Korea and suggests that farm animals may act as reservoirs for zoonotic tick-borne pathogens.

Keywords: *Anaplasma*, *Bartonella*, *Borrelia*, *Haemaphysalis longicornis*, North Korea

Ehrlichia chaffeensis Infection in Dogs in South Korea

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ABSTRACT

Ehrlichia chaffeensis is one of the causative agents of canine ehrlichiosis and human monocytic ehrlichiosis (HME). Canine ehrlichiosis caused by *E. chaffeensis* was diagnosed in two dogs in South Korea based on clinical findings, and the diagnosis was confirmed by polymerase chain reaction (PCR) and DNA sequencing. A 5-year-old intact male American Pit bull terrier allowed outdoors was found to be concurrently infected with *Babesia gibsoni* and *E. chaffeensis*. The major clinical findings were lethargy and reddish urine, and laboratory analysis revealed severe hematuria and thrombocytopenia. In addition, a 3-year-old neutered male Shih-tzu was also found to be infected with *E. chaffeensis*. Although this dog was an indoor companion animal, he was frequently allowed outside for exercise. The clinical signs observed in this dog included generalized purpura with petechiae and ecchymoses due to thrombocytopenia. A 390-bp partial portion of *E. chaffeensis* 16S rRNA gene was amplified in both cases, and nucleotide sequence analysis revealed 99% homology of this fragment with other *E. chaffeensis* isolates. These findings demonstrate the presence of *E. chaffeensis* infection in dogs in South Korea, and this is the first report to confirm clinical cases of *E. chaffeensis* infection in dogs. **Key Words:** *Ehrlichia chaffeensis*—Dog—PCR—Korea.

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Serologic and Molecular Detection of *Ehrlichia chaffeensis* and *Anaplasma phagocytophilum* (Human Granulocytic Ehrlichiosis Agent) in Korean Patients

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Sera from 491 Korean patients with acute febrile diseases were tested for *Ehrlichia chaffeensis* and *Anaplasma phagocytophilum* antibodies by indirect immunofluorescence assay (IFA), Western blotting, and TaqMan real-time PCR. Overall, 0.4% of sera reacted with *E. chaffeensis*, and 1.8% reacted with *A. phagocytophilum* in IFAs. This is the first report of detection of antibodies to *A. phagocytophilum* and *E. chaffeensis* in Korea and suggests the presence of *A. phagocytophilum* and *E. chaffeensis* or antigenically similar species.

Detection of Antibodies to *Anaplasma phagocytophilum* and *Ehrlichia chaffeensis* Antigens in Sera of Korean Patients by Western Immunoblotting and Indirect Immunofluorescence Assays

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Received 6 May 2003/Returned for modification 16 June 2003/Accepted 21 July 2003

Two hundred seventy one serum samples from South Korean patients were tested to detect antibodies against *Anaplasma phagocytophilum* (the human granulocytic ehrlichiosis agent) and *Ehrlichia chaffeensis* (the human monocytic ehrlichiosis agent) by indirect fluorescent-antibody assay (IFA) and the Western blot assay. These sera were collected from patients with symptoms of high fever. The rate of seropositivity for *Orientia tsutsugamushi* was 50.9% by IFA at the Public Health & Environmental Research Institute and National Institute of Health in South Korea. By IFA, 30 (11.1%) and 39 (14.4%) of the serum samples reacted with *A. phagocytophilum* and *E. chaffeensis* antigens, respectively. By the Western blot assays, 24 (8.9%) and 29 (10.7%) of the serum samples reacted with purified *A. phagocytophilum* and *E. chaffeensis* protein antigens, respectively. This report strengthens other evidence regarding the presence of *A. phagocytophilum* and *E. chaffeensis* infections in humans in South Korea.

Detection of *Bartonella* species from ticks, mites and small mammals in Korea

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We investigated the prevalence of *Bartonella* infections in ticks, mites and small mammals (rodents, insectivores and weasels) collected during 2001 through 2004, from various military installations and training sites in Korea, using PCR and sequence analysis of 16S rRNA, 23S rRNA and *groEL* heat shock protein genes. The prevalence of *Bartonella* spp. was 5.2% (n = 1,305 sample pools) in ticks, 19.1% (n = 21) in mesostigmatid mites and 13.7% (n = 424 individuals) in small mammals. The prevalence within the family Ixodidae was, 4.4% (n = 1,173) in *Haemaphysalis longicornis* (scrub tick), 2.7% (n = 74) in *H. flava*, 5.0% (n = 20) in *Ixodes nipponensis*, 11.1% (n = 9) in *I. turdus*, 33.3% (n = 3) in *I. persulcatus* and 42.3% (n = 26) in *Ixodes* spp. ticks. In rodents, the prevalence rate was, 6.7% (n = 373) in *Apodemus agrarius* (striped field mouse) and 11.1% (n = 9) in *Eothenomys regulus* (Korean red-backed vole) and in an insectivore, *Crocidura lasiura*, 12.1% (n = 33). Neither of the two weasels were positive for *Bartonella* spp. Phylogenetic analysis based on amino acid sequence of a portion of the *groEL* gene amplified from one *A. agrarius* spleen was identical to *B. elizabethae* species. We demonstrated the presence of *Bartonella* DNA in *H. longicornis*, *H. flava* and *I. nipponensis* ticks, indicating that these ticks should be added to the growing list of potential tick vectors and warrants further detailed investigations to disclose their possible roles in *Bartonella* infection cycles.

ORIGINAL ARTICLE

Prevalence, Isolation and Molecular Characterization of *Bartonella* Species in Republic of Korea

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Keywords:

striped field mice; *Apodemus agrarius*; *Bartonella*; prevalence; isolation; Republic of Korea

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Summary

To determine the prevalence of *Bartonella* species and identify which species of *Bartonella* naturally infects the striped field mouse (*Apodemus agrarius*) in the Republic of Korea (ROK), spleens from 200 mice were assayed by nested polymerase chain reaction (nPCR) targeting the RNA polymerase subunit beta (*rpoB*) gene and the 16S-23S internal transcribed spacer (ITS) region for members of the genus *Bartonella*. Utilizing PCR techniques, the prevalence of *Bartonella* spp. ranged from 31.5% (63/200) to 62.0% (124/200) for the *rpoB* and ITS gene fragments, respectively. The most prevalent species, *Bartonella grahamii*, was assigned to 17 genotypes and closely related to the zoonotic pathogens, *B. taylorii*, *B. tribocorum*, *B. phoceensis* and *B. henselae*, which also were detected. Two *Bartonella* isolates (KRBG28 and KRBG32) were recovered from blood of *A. agrarius* captured in Gyeonggi Province, ROK. Comparison of the 16S rRNA, hemin-binding protein E (*hbpE*), glutamate dehydrogenase 1 (*gdh1*), invasion-associated protein B (*ialB*), cell division protein (*ftsZ*), citrate synthase (*gltA*), 60 kDa heat shock protein (*groEL*), *rpoB* gene fragments and the ITS region sequences from the isolates with GenBank was confirmed as *B. grahamii*. Phylogenetic analysis based on the alignment of concatenated sequences (4933 bp) of KRBG28 and KRBG32 clustered with *B. grahamii*, forming an independent clade between Asian and American/European *B. grahamii* genogroups.

Tick-Borne Rickettsial Pathogens in Ticks and Small Mammals in Korea

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In order to investigate the prevalence of tick-borne infectious agents among ticks, ticks comprising five species from two genera (*Hemaphysalis* spp. and *Ixodes* spp.) were screened using molecular techniques. Ticks (3,135) were collected from small wild-caught mammals or by dragging/flagging in the Republic of Korea (ROK) and were pooled into a total of 1,638 samples (1 to 27 ticks per pool). From the 1,638 tick samples, species-specific fragments of *Anaplasma phagocytophilum* (1 sample), *Anaplasma platys* (52 samples), *Ehrlichia chaffeensis* (29 samples), *Ehrlichia ewingii* (2 samples), *Ehrlichia canis* (18 samples), and *Rickettsia rickettsii* (28 samples) were amplified by PCR assay. Twenty-one pooled and individual tick samples had mixed infections of two (15 samples) or three (6 samples) pathogens. In addition, 424 spleen samples from small captured mammals (389 rodents, 33 insectivores, and 2 weasels) were screened for selected zoonotic pathogens. Species-specific DNA fragments of *A. phagocytophilum* (110 samples), *A. platys* (68 samples), *E. chaffeensis* (8 samples), *E. ewingii* (26 samples), *E. canis* (51 samples), and *Rickettsia* sp. (22 samples) were amplified by PCR assay. One hundred thirty small mammals had single infections, while 4, 14, and 21 striped field mice (*Apodemus agrarius*) had mixed infections of four, three, and two pathogens, respectively. Phylogenetic analysis based on nucleotide sequence comparison also revealed that Korean strains of *E. chaffeensis* clustered closely with those from China and the United States, while the *Rickettsia* (rOmpA) sequences clustered within a clade together with a Chinese strain. These results suggest that these agents should be considered in differential diagnosis while examining cases of acute febrile illnesses in humans as well as animals in the ROK.

Microbial pathogens in ticks, rodents and a shrew in northern Gyeonggi-do near the DMZ, Korea

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A total of 1,618 ticks [420 individual (adults) and pooled (larvae and nymphs) samples], 369 rodents (*Apodemus agrarius*, *Rattus norvegicus*, *Tscherskia triton*, *Mus musculus*, and *Myodes regulus*), and 34 shrews (*Crocidura lasiura*) that were collected in northern Gyeonggi-do near the Demilitarized Zone (DMZ) of Korea during 2004–2005, were assayed by PCR for selected zoonotic pathogens. From a total of 420 individual and pooled tick DNA samples, *Anaplasma* (A.) *phagocytophilum* (16), *A. platys* (16), *Ehrlichia* (E.) *chaffeensis* (63), *Borrelia burgdorferi* (16), and *Rickettsia* spp. (198) were detected using species-specific PCR assays. Out of 403 spleens from rodents and shrews, *A. phagocytophilum* (20), *A. platys* (34), *E. chaffeensis* (127), and *Bartonella* spp. (24) were detected with species-specific PCR assays. These results suggest that fevers of unknown causes in humans and animals in Korea should be evaluated for infections by these vector-borne microbial pathogens.

Keywords: *Bartonella*, *Borrelia*, *Rickettsia*, rodents, *Crocidura lasiura*, tick-borne pathogens

ORIGINAL ARTICLE

Korean J Pediatr 1994 March;37(3) :356-367.

***Coxiella burnetii* Infection In Patients With Various Diseases**

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ABSTRACT

Coxiella burnetii (*C. burnetii*) was first recognized as the agent of **Q fever** in 1937. Q fever is an acute self-limited febrile illness. However, it manifests with several clinical symptoms depending upon the organs that are involved. The association of *C. burnetii* with human neoplasia has been rarely reported. We prospectively studied the 55 patients with fever of unknown origin, pneumonia, hepatosplenomegaly, lymphadenopathy, leukemia, lymphoma, and immunodeficiency and 14 persons who contacted the Q fever patients. The patient's sera were tested for antibodies specific for *C. burnetii*, using indirect fluorescent antibody techniques (IFA). 1) We serologically confirmed 23 *C. burnetii* infection. The 23 children with Q fever ranged in age from 0 to 15 years, with mean age of 4 years 11 months. Seventeen were boys and 6 were girls. 2) Characteristic symptoms and signs were fever (9/12 cases), rash (8/12 cases), hepatosplenomegaly (8/8 cases) and lymphadenopathy (14/27 cases). Five cases among 14 asymptomatic cases who contacted Q fever patients showed positive IFA test. One suffered from irregular uterine contraction, 4 weeks after contact with a Q fever patient. 3) There were no history of exposure to domestic animal carriers or contaminated dust, or drinking raw milk except one family. Three attending doctors and her father infected by a patient with Q fever. These suggested the person to person transmission of Q fever in a family and house staffs infected by a patient of Q fever. 4) Q fever (9 cases), acute lymphoblastic leukemia (2 cases), acute myelomonocytic leukemia (1 case), hairy cell leukemia (1 case), Kawasaki disease (4 cases) and congenital dyserythropoietic anemia (1 case) showed positive IFA test. 5) Of 9 cases who suffered from only Q fever, 7 cases were confirmed hairy cell formation in their peripheral blood. One case was diagnosed as hairy cell leukemia after bone marrow study. Of 7 cases who showed hairy cells, all had hepatomegaly, 6 cases had lymphadenopathy and 5 cases showed splenomegaly. All except 1 case who was not followed cured after treatment. 6) We treated Q fever patients with rifampin and/or ciprofloxacin, and/or tetracycline (over 8 year-old of age) for 2-4 weeks. One 25 month-old patient with hairy cell leukemia was treated with rifampin, ciprofloxacin and tetracycline for 4 weeks, and rifampin for 8 months. A pregnant patient was administered with rifampin, and treated with rifampin and ciprofloxacin after delivery. We gave rifampin in one newborn baby. In conclusion, we suggest that Q fever should be considered in the differential diagnosis of patients with FUO, hepatosplenomegaly and/or immunodeficiency.

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Seroprevalence of *Coxiella burnetii* in cattle and farm-raised deer in Korea

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Q fever, caused by *Coxiella burnetii*, is a worldwide zoonosis that affects not wild animals but domestic animals throughout the world, except in New Zealand. Domestic ruminants are considered to be a major infection source of Q fever in humans. However, few studies on the prevalence of Q fever in humans or animals in Korea have been conducted. Thus, the aim of this study was to investigate the seroprevalence of Q fever in meat cattle and deer. Blood samples were collected from 1634 ruminants: 1000 cattle, 604 wapiti, and 30 sika deer. The blood samples were analyzed with CHEKIT Q fever ELISA kits. Thirteen of 1000 (1.3%) cattle, 10 of 604 (about 1.7%) wapiti, and 0 of 30 (0%) sika deer had antibodies against *C. burnetii*. The prevalence of Q fever in this study was quite low. However, the public health implications of these findings are important, because they indicate that seropositive animals that are asymptomatic may be shedding *C. burnetii* consistently. This condition could increase the risk of Q fever infection in Korea, especially because many Koreans habitually consume raw meat and drink deer blood.

Microbiol. Immunol., 48(2), 125–130, 2004

Identification of the *Coxiella* sp. Detected from *Haemaphysalis longicornis* Ticks in Korea

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Abstract: Two *Haemaphysalis longicornis* ticks were found positive in PCR assay of *com-1* gene to detect *Coxiella burnetii* DNA from 100 ticks. The nucleotide sequences of *com-1* and 16S rRNA gene were determined from 2 ticks and compared with those of other *C. burnetii* strains. The results suggest that *H. longicornis* harbor *Coxiella* sp. bacteria in Korea. Furthermore, *icd*, *cbhE'*, and *cbbE'* genes are *C. burnetii* specific genes whereas *com-1* gene is *Coxiella* genus specific gene. This study gives the first documentation to prove the existence of *Coxiella* sp. in tick collected in Korea.

J Korean Med Sci 2006; 21: 823-6. ISSN 1011-8934

Seroprevalence of *Coxiella burnetii* Infection in Dairy Cattle and Non-symptomatic People for Routine Health Screening in Korea

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We report results on the seroprevalence of antibodies to *Coxiella burnetii* in cattle and healthy people in Korea. Upon agreement with dairy owners, serum samples from 414 dairy cattle were collected between March and June 2001 and samples from 205 people for health screening were collected between April and December 2002. The sera were analyzed for the presence of anti-*C. burnetii* phase II antibodies using an indirect microimmunofluorescence test; strong fluorescence at a 1:32 dilution was regarded as positive. The overall seroprevalence of *C. burnetii* in cattle in Korea was 25.6%, with regional variation from 8.9 to 59.3%. Of the positive serum samples, 75.5% had antibody titers $\geq 1:256$. By contrast, only 1.5% of people in a rural area were seropositive, and most of the positive samples had low antibody titers. In conclusion, this study showed that relatively high seropositivity of *C. burnetii* in dairy cattle, accordingly, the studies on the high-risk groups are needed to evaluate the seroprevalence for this organism in Korea.

NOTE *Wildlife Science*

The Prevalence of *Coxiella burnetii* Infection in Wild Korean Water Deer, Korea

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ABSTRACT. The aim of this study was to evaluate the prevalence of *Coxiella burnetii* infection in wild Korean water deer (*Hydropotes inermis argyropus*) in Korea, by using serology and real-time PCR analyses. One hundred ninety-six sera were collected from 4 provinces and tested for anti-*C. burnetii* antibody detection, by means of CHEKIT Q fever ELISA kit; and *C. burnetii* IS1111 insertion sequence detection, by means of real-time PCR. Antibodies were detected in 18 of the 196 (9.18%) serum samples, whereas genomes of *C. burnetii* were detected in 13 of the 196 (6.63%) serum samples. Based on overall high seroprevalence, the public health implications of these findings are important, because they indicate that asymptomatic seropositive or seronegative wild animals may be consistently shedding *C. burnetii*. This is the first study of *C. burnetii* prevalence in Korean water deer in the Republic of Korea that has indicated the presence of infected animals throughout the country.

KEY WORDS: *Coxiella burnetii*, prevalence, Q fever, wild Korean water deer.

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Dispatch

Human Granulocytic Anaplasmosis, South Korea, 2013

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[Suggested citation for this article](#)

Abstract

We report a patient with human granulocytic anaplasmosis in South Korea. The patient had fever and thrombocytopenia. Human granulocytic anaplasmosis was confirmed by seroconversion, PCR, and sequence analysis for *Anaplasma phagocytophilum*. Morulae were observed in the cultured HL-60 cells inoculated with blood from the patient.

On This Page

[The Study](#)

[Conclusions](#)

[Suggested Citation](#)

Figures

[Figure 1](#)

[Figure 2](#)

Tables

Ann. N.Y. Acad. Sci. 990: 118–125 (2003).

Molecular Epidemiological Study for Tick-Borne Disease (*Ehrlichia* and *Anaplasma* spp.) Surveillance at Selected U.S. Military Training Sites/Installations in Korea

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ABSTRACT: Vector-borne diseases are a potential public health threat to U.S. Forces Korea (USFK). *Ehrlichia* and *Anaplasma* spp., transmitted by ticks, are only two of several diseases that may affect military readiness and operations. Rodents were collected at selected U.S. military installations and training sites in the Republic of Korea. DNA was extracted from spleen tissues and assayed by PCR methods for *Ehrlichia* and *Anaplasma* species. From rodents and mustelids collected during 1999 and 2000, a total of 196 *Apodemus agrarius* (striped field mouse), 2 *Mustela sibirica* (weasel), and 1 *Cricetulus triton nestor* (Korean greater long-tailed hamster) were assayed for *Ehrlichia* and *Anaplasma* species-specific DNA fragments. Rodent surveillance indicated a very high prevalence of *Ehrlichia* and *Anaplasma* spp. at selected training sites. *Ehrlichia/Anaplasma* DNA were identified from spleen tissue from 157 *Apodemus agrarius*, 1 *Mustela sibirica*, and 1 *Cricetulus triton nestor*. Species-specific DNA fragments of *E. canis* (45), *E. ewingii* (16), *A. phagocytophila* (5), and *A. platys* (62) were amplified by PCR techniques. Seventy-one striped field mice had single infections, while 24 had mixed infections of 2 (17 specimens), 3 (7 specimens), or 4 (1 specimen) pathogens. The striped field mouse plays a role as a reservoir for latent infections of various *Ehrlichia* or *Anaplasma* species.

KEYWORDS: *Ehrlichia* sp.; *Anaplasma* sp.; rodents; Korea; military personnel

J Vet Clin 26(6) : 515-519 (2009)



Detection of Antibodies Reacting with *Anaplasma phagocytophilum* and *Ehrlichia chaffeensis* from Cats, Horses and Cattle in Korea

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(Accepted: October 13, 2009)

Abstract : Antibodies to *Anaplasma phagocytophilum* and *Ehrlichia chaffeensis* were detected by the immunofluorescent antibody (IFA) test in sera collected from cats, thoroughbred horses and Holstein cattle in Gwangju, Jeonju and Jeju Island of Korea. Two hundred fifty four sera (33 feral and pet cats, 92 grazing horses and 129 grazing cattle) were obtained from Republic of Korea. Antibodies to *A. phagocytophilum* (titer ≥ 80) were detected in 6 of the 33 feral and pet cats (18.2%), and 1 seropositive cat (3.0%) also had antibodies to *E. chaffeensis*. Only 1 of 129 (0.8%) cattle and 2 of 92 (2.2%) horses had antibodies to *A. phagocytophilum*. Antibodies to *E. chaffeensis* were not detected in either of these animals. This is the first report of serological evidence of *A. phagocytophilum* and *E. chaffeensis* from cats, cattle and horses in Korea. These rickettsial agents could have an important impact on human health or impact animal health with economic losses among industrial grazing animals in Korea.

Genetic Identification and Phylogenetic Analysis of *Anaplasma* and *Ehrlichia* Species in *Haemaphysalis longicornis* Collected from Jeju Island, Korea

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A total of 1,395 *Haemaphysalis longicornis* ticks collected from Jeju Island of Korea were examined by 16S rRNA gene-based nested PCR for the presence of infection with *Anaplasma* and *Ehrlichia* species. Template DNAs to detect the tick-borne pathogens were prepared from a total 506 tick pools. Eight genera of *Anaplasma* and six *Ehrlichia* by 16S rRNA gene PCR and sequencing analysis were identified. *A. phagocytophilum* was the most prevalent (27 [1.9%]) by nested PCR, followed by *A. bovis* (5 [0.4%]), *E. chaffeensis* (4 [0.2%]), and *A. centrale* (1 [0.1%]). In the phylogenetic analysis based on 16S rRNA sequences, eight genera of *Anaplasma* group (> 99.4% homology) and six *Ehrlichia* group (> 99.5% homology) were close to deposited *A. marginale* strains (AF309867, AF414874, and FJ226454) and *Ehrlichia* sp. (DQ324547), respectively. Three *Anaplasma* species groups *A. phagocytophilum* (group A), *A. bovis* (group B), and *A. centrale* (group C) and one *Ehrlichia* species *E. chaffeensis* (group D) were determined by comparing with *Anaplasma* and *Ehrlichia* related sequences. First, twenty-eight *A. phagocytophilum* clones belonging to group A were divided into 7 genotypes. The sequence similarity among genotypes A1 to A4 was very high (> 99.6%). Genotype B2 was close to *A. bovis* from Korea (99.7%). Genotype D1 was close to known *E. chaffeensis* strains (M73222, AF147752, and AY350424) and their similarity value was 99.7%. In conclusion, the genera of *Anaplasma*/*Ehrlichia*, *A. phagocytophilum*, and *E. chaffeensis* identified in predominant *H. longicornis* ticks were ubiquitous throughout the Jeju Island. The various native groups have been found through sequence identities and phylogenetic analysis.

Key Words: *Haemaphysalis longicornis*, Tick-borne pathogens, *Anaplasma phagocytophilum*, *Ehrlichia chaffeensis*

Molecular Detection of *Ehrlichia chaffeensis* and *Anaplasma bovis* in the Salivary Glands from *Haemaphysalis longicornis* Ticks

Mi-Jin Lee¹ and Joon-Seok Chae²

Abstract

The salivary gland (SG) of tick plays an important role as a route in the dissemination of tick-borne pathogens to their hosts. We evaluated the presence of these pathogens in the SGs of *Haemaphysalis longicornis* ticks, and these ticks were collected from grazing cattle in Jeju Island, Korea. Of total 463 one-side SGs, 56 (12.1%) SGs were positive for *Ehrlichia chaffeensis* and 11 (2.4%) were positive for *Anaplasma bovis*. In addition, two (0.4%) SGs were co-infected with both *E. chaffeensis* and *A. bovis*. In conclusion, we specifically describe the presence of *E. chaffeensis* and *A. bovis* in the SGs of *H. longicornis* ticks in Korea.

New Genetic Variants of *Anaplasma phagocytophilum* and *Anaplasma bovis* from Korean Water Deer (*Hydropotes inermis argyropus*)

Jun-gu Kang,¹ Sungjin Ko,¹ Young-Jun Kim,² Hyo-Jin Yang,² Hang Lee,²
Nam-shik Shin,³ Kyoung-seong Choi,⁴ and Joon-seok Chae¹

Abstract

Wild deer are one of the important natural reservoir hosts of *Anaplasma* species, which cause granulocytic anaplasmosis in equines, canines, and humans. The objective of the present study was to determine whether and what species of *Anaplasma* naturally infect Korean water deer (KWD) in the Republic of Korea. A total of 66 spleens from KWD carcasses were collected by the Conservation Genome Resource Bank for Korean Wildlife in Korea between March 2008 and May 2009. Polymerase chain reaction (PCR) was performed using 16S ribosomal (r)RNA, with *ankA*, *groEL*, and *msp2* gene primers to amplify the genes of *Anaplasma* and *Ehrlichia*. Using 16S rRNA-based nested PCR, *Anaplasma phagocytophilum* and *Anaplasma bovis* were detected in 42 (63.6%) and 23 (34.8%) of 66 KWD spleens, respectively. The 42 *A. phagocytophilum* were classified into five genotypes and the 23 *A. bovis* were classified into two genotypes by sequence analysis. By *ankA*-, *groEL*-, and *msp2*-based nested PCR, *A. phagocytophilum* was detected in 1 (1.5%), 7 (10.6%), and 3 (4.6%) of 66 samples, respectively. These gene sequences had only one genotype. Five of seven obtained 16S rRNA gene sequences have never been identified. The *ankA*, *groEL*, and *msp2* obtained gene sequences represented new genotypes. This is the first report of *A. phagocytophilum* and *A. bovis* in KWD, suggesting that they may act as reservoirs for anaplasmosis zoonotic pathogens.

BRIEF COMMUNICATION

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Molecular detection of *Anaplasma bovis* in Holstein cattle in the Republic of Korea

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Abstract

Anaplasmosis is a tick-borne infectious disease that affects both human and animal health. This study was performed to characterize and investigate the prevalence of infection with *Anaplasma bovis* in Holstein cattle originating from two regions in the Republic of Korea (ROK). Blood samples (n = 151; 80 from Namwon and 71 from Jeju Island) were analyzed by polymerase chain reaction, and the prevalence of *A. bovis* infection was compared before and after grazing. In Namwon, *A. bovis* infection was not detected, while in the Jeju Island, *A. bovis* infection was detected in three of 13 animals after grazing. Phylogenetic analysis revealed that the *A. bovis* isolates had homology (97.1–99.7%) with a Korean spotted deer (*Cervus nippon*) isolate and *Haemaphysalis longicornis* tick isolates identified in the ROK. *A. bovis* infection has not previously been diagnosed in cattle in the ROK. This study shows that *A. bovis* infection in the Jeju Island is closely related to grazing.

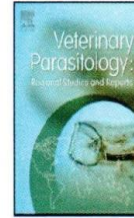
Keywords: *Anaplasma bovis*, Grazing, Holstein cattle, Ticks



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Molecular identification of selected tick-borne pathogens in wild deer and raccoon dogs from the Republic of Korea

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ABSTRACT

In this study, we investigated tick-borne pathogens in blood samples collected from 34 wild animals from six different regions in the Republic of Korea, including 18 Korean water deer (*Hydropotes inermis argyropus*), 15 Korean raccoon dogs (*Nyctereutes procyonoides koreensis*), and one roe deer (*Capreolus capreolus*). Polymerase chain reaction revealed *Anaplasma* spp. infection in 11 Korean water deer, one roe deer, and one Korean raccoon dog. *Theileria* spp. infection was detected in 14 Korean water deer and one roe deer. Co-infection with two pathogens (*Anaplasma* spp. and *Theileria* spp.) was identified in 10 Korean water deer and one roe deer. *Ehrlichia* and *Rickettsia* spp. infections were not detected in any of the animals. Genetic analysis showed that *Anaplasma* sp., *A. bovis*, *A. phagocytophilum*, *Theileria* sp., *T. cervi*, and *T. luwenshuni* were present in these animals. Our results showed that *T. luwenshuni* was the most prevalent species found in Korean water deer. Interestingly, our findings demonstrated that the Korean raccoon dog was a reservoir for anaplasmosis, indicating that transmission of *A. bovis* was not restricted to host species. The roe deer was found to be infected with a type F *T. cervi* strain. To the best of our knowledge, this study is the first to report *A. bovis* infection in Korean raccoon dogs, *T. cervi* in roe deer, and *T. luwenshuni* in Korean water deer. Our results indicated that wild animals represent possible reservoirs for these tick-borne pathogens, thus playing an important role in the transmission of tick-borne diseases (TBDs) in domestic animals, livestock, and humans. Furthermore, our findings highlight the risk associated with introducing new pathogens as well as the role of wild animals in the transmission and spread of these zoonotic TBD pathogens.

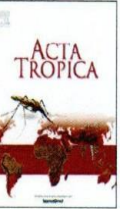
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First report of *Anaplasma phagocytophilum* infection in Holstein cattle in the Republic of Korea

Du-Gyeong Han^a, Ji-Hyoung Ryu^a, Jeong-Byoung Chae^b, Dong-Woo Kim^a, Chan-Ho Kwon^a, Kyoung-Seong Choi^{a,*}



Abstract

Global warming has increased the incidence and risk of tick-borne diseases in domestic animals and humans in the Republic of Korea (ROK). In this study, we investigated the prevalence of *Anaplasma phagocytophilum* in Holstein cattle (n = 214) in the ROK using specific PCR assays. *A. phagocytophilum* infection was detected in only two animals (0.93%, 2/214). Our findings showed that PCR assay using the 16S rRNA gene, but not groEL, was suitable for detection of *A. phagocytophilum* in cattle. Phylogenetic analysis based on the 16S rRNA gene showed that *A. phagocytophilum* was divided into two clades. Clade 1 included Korean isolates, such as those from dogs, cats, Korean water deer, and ticks, while *A. phagocytophilum* identified in Holstein cattle formed clade 2. Our results suggest that there is genetic variability among isolates of *A. phagocytophilum* circulating in the ROK. This is the first study to report *A. phagocytophilum* infection in Holstein cattle in the ROK. As *A. phagocytophilum* has zoonotic potential, additional epidemiological studies are needed to investigate the prevalence and genetic characterization of *A. phagocytophilum* from different regions and hosts.

ORIGINAL ARTICLE

Hematological Changes Associated with *Theileria orientalis* Infection in Korean Indigenous Cattle

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Abstract: Tick-borne pathogens can cause serious problems in grazing cattle. However, little information is available on tick-mediated diseases in cattle grazing on mountains. Thus, this study aimed to understand the potential problems related to tick-borne diseases in grazing cattle through the investigation of prevalent tick-transmitted infections, and their associated hematological changes, in terms of season and grazing type in Korean indigenous cattle (= Hanwoo). Hanwoo cattle from 3 regions of the Republic of Korea (= Korea) were either maintained indoors or placed on grassy mountains from spring to fall of 2014 and 2015. Cattle that grazed in mountainous areas showed a greater prevalence of tick-borne infections with an increased *Theileria orientalis* infection rate (54.7%) compared to that in non-grazing cattle (16.3%) ($P < 0.001$). Accordingly, the red blood cell (RBC) count and hematocrit (HCT) values of grazing cattle were significantly lower than those of non-grazing cattle throughout the season ($P < 0.05$). Moreover, RBC, hemoglobin (Hb), and HCT of *T. orientalis*-positive group were significantly lower than those of *T. orientalis*-negative group ($P < 0.05$). *T. orientalis* is a widespread tick-borne pathogen in Korea. Grazing of cattle in mountainous areas is closely associated with an increase in *T. orientalis* infection (RR=3.4, $P < 0.001$), and with consequent decreases in RBC count and HCT. Thus, these findings suggest that the Hanwoo cattle in mountainous areas of Korea are at a high risk of infection by *T. orientalis*, which can lead to hematological alterations. This study highlights the necessity of preventive strategies that target *T. orientalis* infection.

Key words: *Theileria orientalis*, grazing, Hanwoo cattle, RBC profile, tick-borne pathogen

BRIEF COMMUNICATION

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Pathogenic genotype of major piroplasm surface protein associated with anemia in *Theileria orientalis* infection in cattle

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Abstract

Serious disease outbreaks in cattle caused by *Theileria orientalis* have emerged in the Asia-Pacific region. Genetic variables of the major piroplasm surface protein (MPSP) expressed on the surface of the piroplasm inside *T. orientalis*-infected erythrocytes are considered to be associated with variation in the pathogenicity of *T. orientalis*. Our study describes the clinically relevant MPSP types associated with anemia in *Theileria*-infected cattle. These results revealed that MPSP expression plays an important role in hematological alterations in *Theileria*-infected cattle, and that MPSP type 1 is strongly associated with bovine anemia, which can be a potential target for the prevention of bovine theileriosis.

Keywords: Anemia, Major piroplasm surface protein, MPSP type 1, *Theileria orientalis*



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Seasonal changes in hemograms and *Theileria orientalis* infection rates among Holstein cattle pastured in the mountains in the Republic of Korea

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ABSTRACT

In the current study, we compared seasonal changes in complete blood counts (CBCs) and rates of infection with a tick-borne pathogen between Holstein cattle housed indoors and those maintained outside on pasture. There were differences in white blood cell (WBC) parameters, but the changes were not associated with seasons or the housing type. Analysis of red blood cell (RBC) parameters showed lower values in August and November versus March, and in the cattle maintained on pasture versus the housed cattle. In comparison with the RBC count of the housed cattle in March (10.1 M/ μ L), the RBC counts of the pastured cattle were significantly lower in August (7.8 M/ μ L; $p < 0.01$) and November (7.5 M/ μ L; $p < 0.01$). The hematocrit (HCT) also showed a decrease in March (33.5%), August (30.0%, $p < 0.01$) and November (28.5%, $p < 0.01$). According to PCR analysis, the *Theileria* infection rate among the pastured cattle in March was only 11%, but this rate increased to 22% and 60% in August and November, respectively. The RBC count (7.4 M/ μ L) and HCT (27.7%) values in *Theileria*-positive pastured cattle in November showed a dramatic decrease compared to those of cattle examined in March. Phylogenetic analysis revealed that these *Theileria* isolates correspond to *T. orientalis*. These results suggest that a remarkable increase in tick infestation in mountainous areas in the summer may cause increased rates of infection with *T. orientalis*, leading to significant changes in the RBC profile after grazing. Therefore, these hematological changes may be associated with *T. orientalis* infection caused by tick-biting; thus, additional studies on the pathogenicity of *T. orientalis* are needed.

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SHORT COMMUNICATION

Genetic characterization of *Theileria orientalis* from cattle in the Republic of Korea

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Abstract

Theileria orientalis is the causative agent of benign theileriosis, which is distributed mainly in Asian countries, and causes serious economic losses in the livestock industry. The present study was performed to investigate the epidemiology of *T. orientalis* infections in cattle in the Republic of Korea (ROK) and to characterize the genetic diversity of *T. orientalis* based on the major piroplasm surface protein (MPSP) genes. In 2015, between July and August, blood samples were collected from 138 asymptomatic cattle in four different geographical regions (Hoengseong, Jeongeup, Namwon, and Jeju island) in the ROK. In total, 57 blood samples (41.3 %, 57/138) were positive for *T. orientalis* based on PCR amplification of the MPSP gene. A high prevalence of *T. orientalis* infection was observed in Jeju island, whereas the infection rate was relatively low in Jeongeup. Phylogenetic analysis showed that isolates identified in this study belonged to four MPSP genotypes, specifically types 1, 2, 3, and 7. The distribution of the four genotypes varied considerably among the four regions; types 1, 2, and 3 were detected in Jeju island, whereas types 1 and 7 were found in Namwon, types 1 and 2 in Jeongeup, and type 2 in Hoengseong. To our knowledge, this is the first report on the identification of type 7 *T. orientalis* in cattle in the ROK. These results suggest that the MPSP genotypes detected in this study showed genetic diversity related to geographical location. Our findings revealed that the *T. orientalis* infection rate was relatively high, indicating that *T. orientalis* infection is closely associated with grazing. Of the four MPSP genotypes, the prevalence of the most pathogenic type 2 was relatively high in the ROK. Therefore, further studies should focus on the development of an effective monitoring and prevention program for *T. orientalis*.

■ BRIEF COMMUNICATION

Detection of Tick-Borne Pathogens in the Korean Water Deer (*Hydropotes inermis argyropus*) from Jeonbuk Province, Korea

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Abstract: The objective of this study was to investigate the prevalence of tick-borne pathogens in the Korean water deer (*Hydropotes inermis argyropus*). Pathogens were identified using PCR which included *Anaplasma*, *Ehrlichia*, *Rickettsia*, and *Theileria*. *Rickettsia* was not detected, whereas *Anaplasma*, *Ehrlichia*, and *Theileria* infections were detected in 4, 2, and 8 animals, respectively. The most prevalent pathogen was *Theileria*. Of the 8 *Theileria*-positive animals, 2 were mixed-infected with 3 pathogens (*Anaplasma*, *Ehrlichia*, and *Theileria*) and another 2 animals showed mixed-infection with 2 pathogens (*Anaplasma* and *Theileria*). Sequencing analysis was used to verify the PCR results. The pathogens found in this study were identified as *Anaplasma phagocytophilum*, *Ehrlichia canis*, and *Theileria* sp. To the best of our knowledge, this is the first report identifying these 3 pathogens in the Korean water deer. Our results suggest that the Korean water deer may serve as a major reservoir for these tick-borne pathogens, leading to spread of tick-borne diseases to domestic animals, livestock, and humans. Further studies are needed to investigate their roles in this respect.

THE END