



**Prevalence of Canine Ehrlichial  
and Borrelial Infection  
in GSDs of Korea**

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# Introduction



- Emerging various tick-borne infectious disease
- Worldwide geographical distribution
- Possible infection of ehrlichia spp. and borrelia spp. transmitted by various species of ticks between human and animal
- No broad survey reports in dogs of Korea

# Objective



- Survey prevalence of canine ehrlichial and borrelial infection in outdoor German Shepherd dogs of Korea
- Identify the molecular DNA fragments of ehrlichia spp. and borrelia spp. by PCR, cloning, sequencing and phylogenetic analysis

# Materials and Methods

- 291 GSDs from different provinces of Korea
  - Canine whole blood collection
  - Period : October 2005 to September 2006
- Complete blood count test
  - MS9-5 Hematology Analyzer, France

# Materials and Methods

- Serological screening test
  - Snap® 3DX assay (ELISA, IDEXX, USA)
  - Ehrlichiosis : sensitivity 98.9 % , specificity 98.2 %
  - Borreliosis : sensitivity 95.0 %, specificity 99.9 %
- Polymerase chain reaction
  - Positive control : *E. chaffeensis* (AY350424)
  - Primary and Nested PCR

# Materials and Methods

## ● Oligonucleotide primers for the detection of tick-borne agents

Target species (Expected size)	Primer name	Sequence (5`-3`)	Annealing temp
<i>Ehrlichia</i> spp (450 bp)	ECC ECB	AGAACGAACGCTGGCGGCAAGC CGTATTACCGCGGCTGCTGGCA	65 °C
<i>E. chaffeensis</i> (390 bp)	HE1 HE3	CAATTGCTTATAACCTTTTGGTTATAAAT TATAGGTACCGTCATTATCTTCCCTAT	55 °C
<i>E. canis</i> (365 bp)	ECAN5 HE3	CAATTATTTATAGCCTCTGGCTATAGGA TATAGGTACCGTCATTATCTTCCCTAT	55 °C
<i>E. ewingii</i> (350 bp)	EE52 HE3	CGAACAATTCCTAAATAGTCTCTGAC TATAGGTACCGTCATTATCTTCCCTAT	55 °C
<i>Borrelia burgdorferi</i> (597 bp)	BBOSPF BBOSPR	AAAGAATACATTAAGTGCGATATT GGGCTTGTAAGCTCTTTAACTG	54 °C

\* Murphy, G.L. et al., 1998. J Vet Parasitol 79:325-339

# Materials and Methods

## PCR for *Ehrlichia spp.*

### First step :

94°C for 3 min (pre-denaturation)

94°C for 1 min

65°C for 2 min

72°C for 2 min

} 30 cycles

72°C for 10 min (post-extension)

### Second step :

94°C for 1 min

55°C for 2 min

72°C for 1.5 min

92°C for 1.5 min

} 2 cycles

55°C for 2 min

72°C for 1.5 min

} 36 cycles

72°C for 10 min

PCR machine: Takara PCR Thermal cycler (TP600, Japan)

# Materials and Methods

- Electrophoresis
  - 1 % agarose gel, 100V for 20min, 100 bp marker
  - UV transilluminator  
(Digital Gel Documentation system,UVT-260D, USA)
- T/A cloning : pGEM-T easy vector
- Sequencing by automatic sequencer  
(ABI 3100 Genetic Analyzer, Bionics, Korea)

# Materials and Methods

- Phylogenetic analysis
  - Nucleotide sequence homology search
    - BLAST network service
    - GENESTREAM network server
    - Multiple sequence alignment
  - Phylogenetic trees
    - Maximum-likelihood (PAUP\*4.0b for Macintosh™)

# Materials and Methods

- **Statistical analysis**

- SPSS (version 12.0)

- $X^2$  (chi-square) test

- Fisher exact test

- Confidence intervals 95 %

- $P < 0.05$  : significant

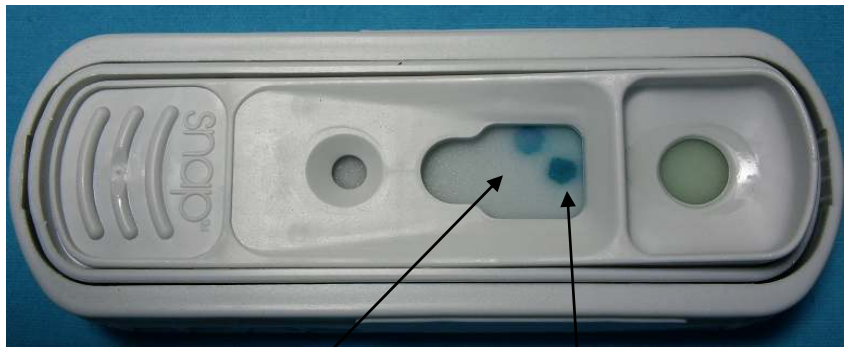
# Results

## ● Result of Complete Blood Count test

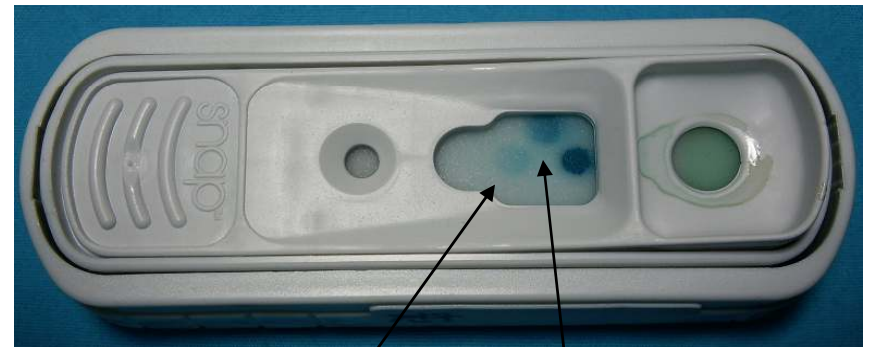
<b>Classified</b>	<b>Platelet count ( x 10<sup>4</sup>/ul)</b>	<b>3DX (+)</b>	<b>PCR (+)</b>	<b>Reference</b>
<b>Severe thrombocytopenia</b>	<b>PLT &lt; 10</b>	<b>8</b>	<b>5</b>	<b>Total No. = 291</b>
<b>Moderate thrombocytopenia</b>	<b>10 &lt; PLT &lt; 20</b>	<b>6</b>	<b>2</b>	
<b>Normal Range</b>	<b>20 &lt; PLT &lt; 50</b>	<b>7</b>	<b>2</b>	
<b>Total tested of CBC</b>		<b>21</b>	<b>9</b>	
<b>Thrombocytopenia rate (%)</b>		<b>66.7</b>	<b>77.8</b>	

# Results

- **Snap 3DX assay (ELISA, IDEXX, USA)**



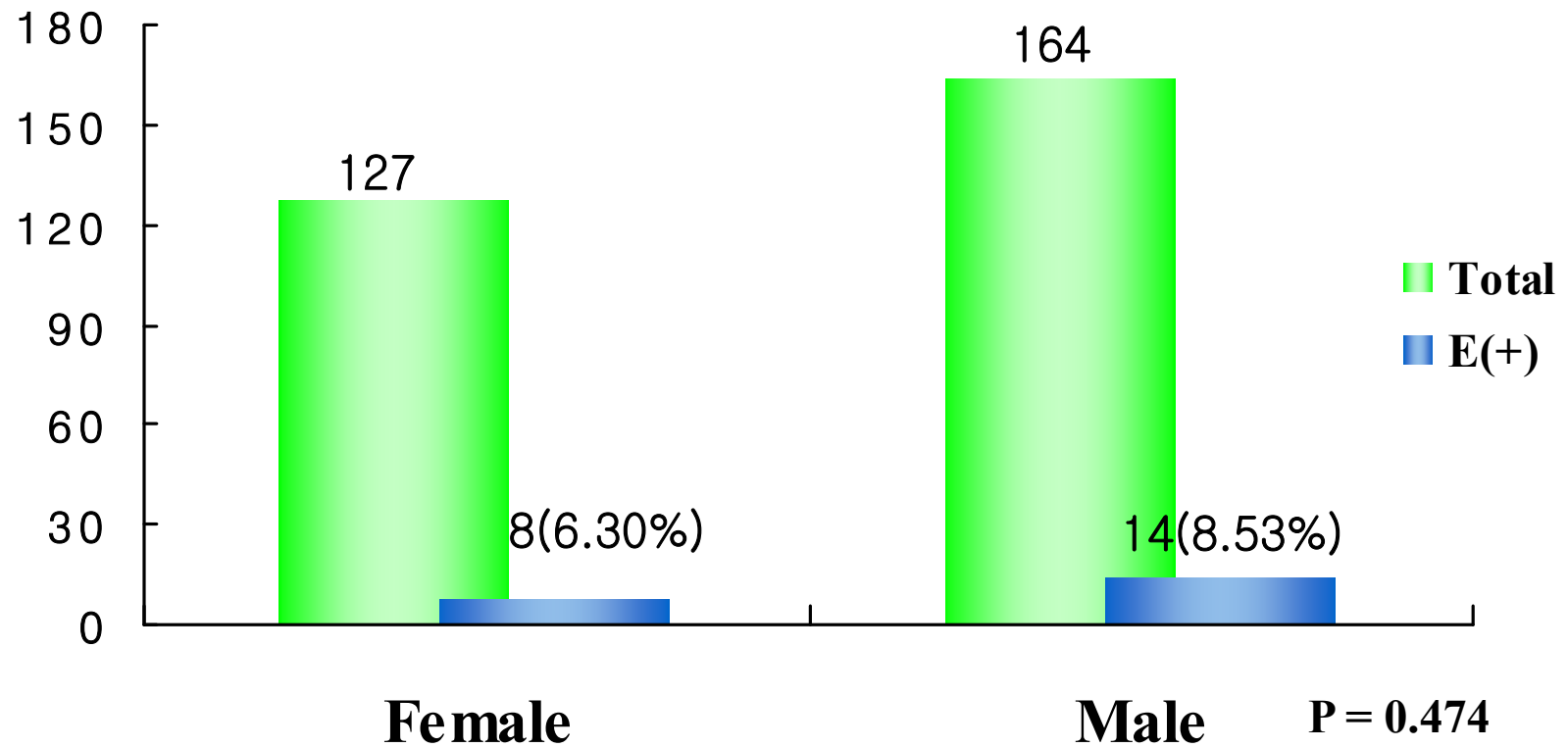
**Ehrlichiosis (+) Control**



**Borreliosis (+) Ehrlichiosis (+)**

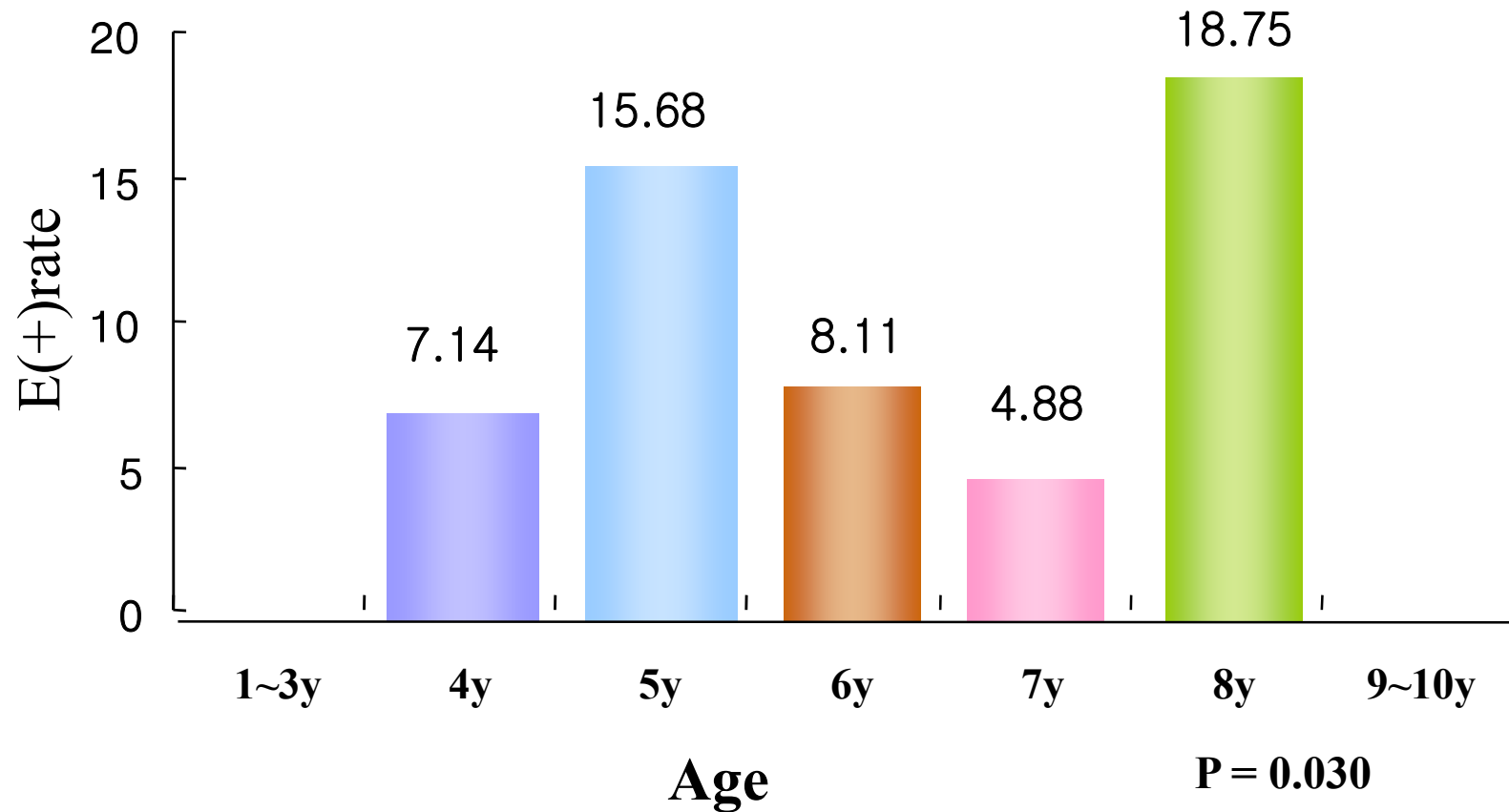
# Results

## ● Sex distribution



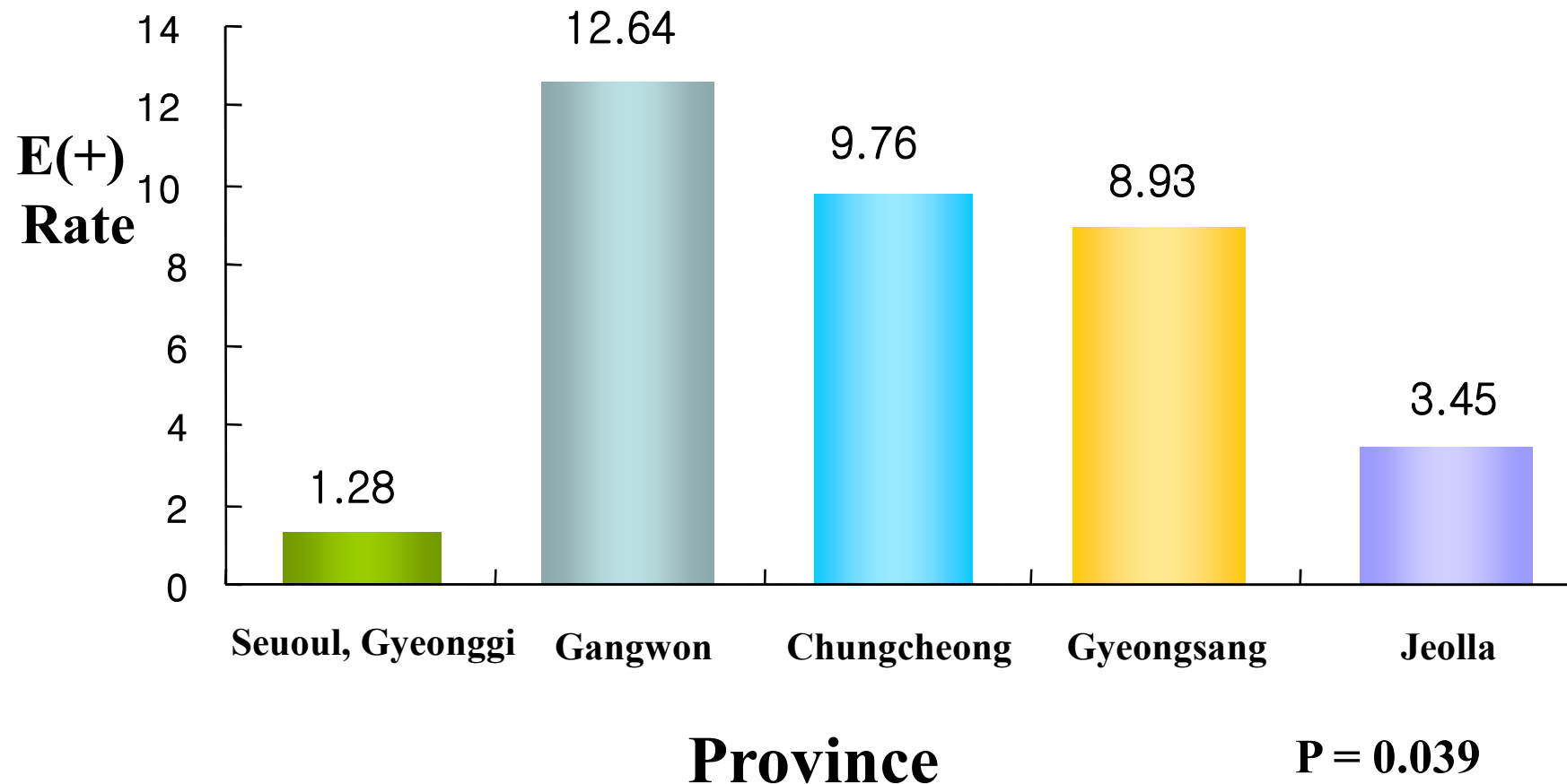
# Results

## ● Age distribution (3DX test)



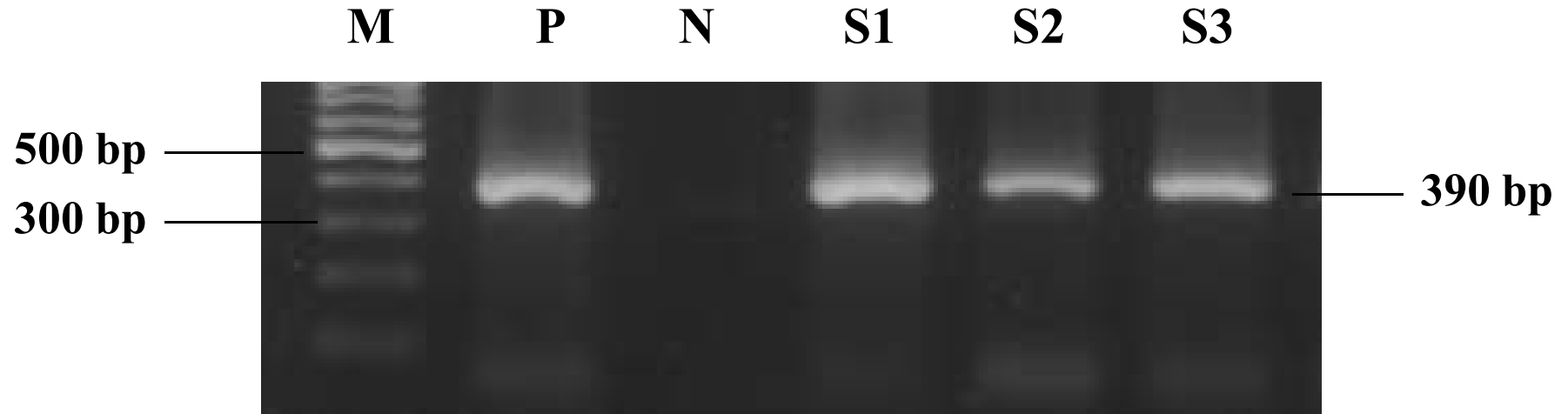
# Results

## ● Geographical distribution



# Results

## ● Result of polymerase chain reaction



Nested PCR showed amplification of 390 bp product for *Ehrlichia chaffeensis*. Lane M, 100bp DNA molecular mass marker; P, positive control; N, negative control; S1~S3, positive samples.

# Results

- Number of positive samples by PCR

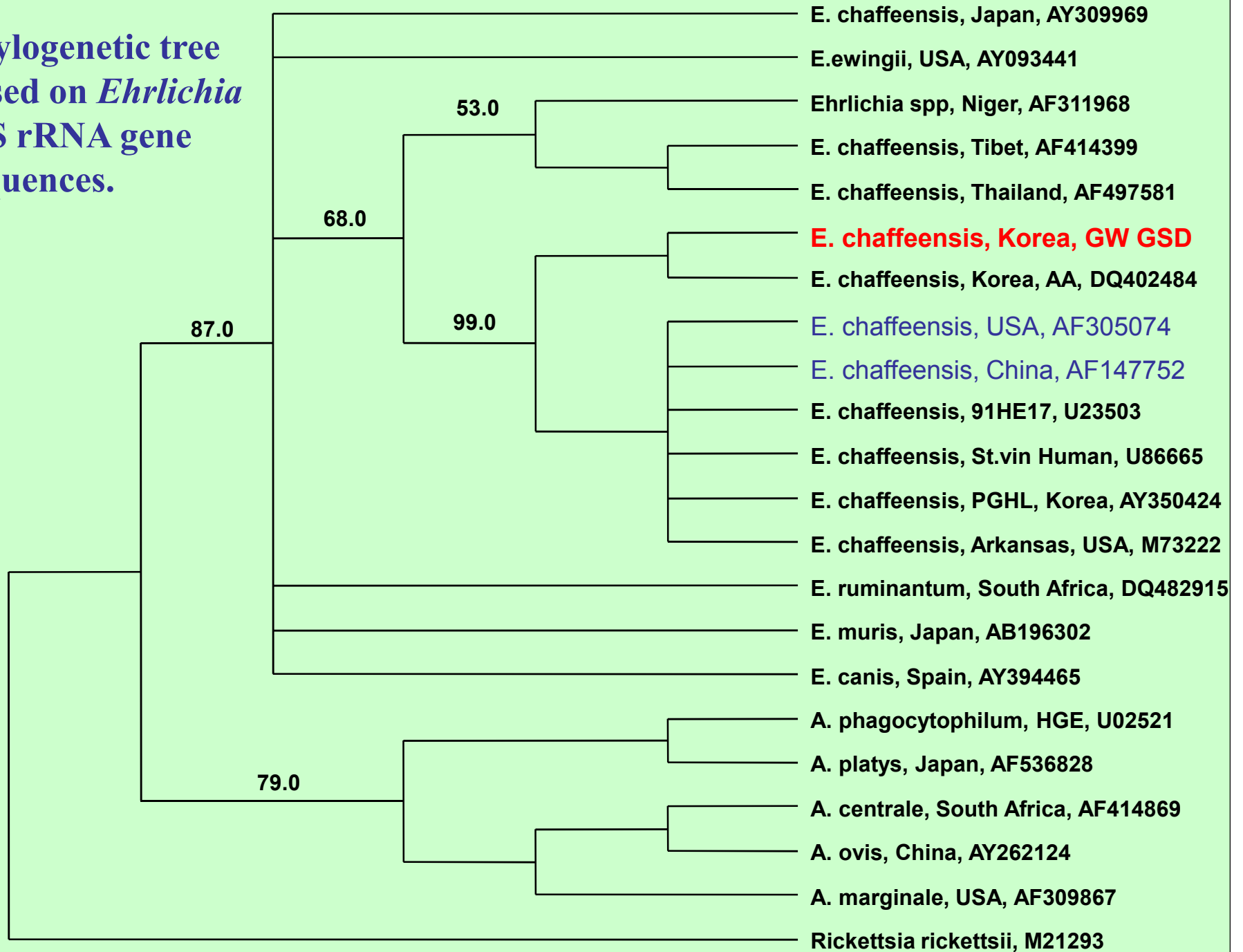
Species	Cases (%)
<i>E. chaffeensis</i>	9 (3.09)
<i>E. canis</i>	0 (0.0)
<i>E. ewingii</i>	0 (0.0)
<i>B. burgdorferi</i>	0 (0.0)

# Results

- **Comparison of 3DX and PCR results**

<b>Classified</b>	<b>3DX(+)</b>	<b>PCR(+)</b>	<b>PCR(+) /3DX(+)</b>
<b>Ehrlichial infection</b>	<b>22/291 (7.56 %)</b>	<b>9/291 (3.09 %)</b>	<b>7/22 ( 31.82 %)</b>
<b>Borrelial infection</b>	<b>5/291 (1.72 %)</b>	<b>-</b>	<b>-</b>

**Phylogenetic tree  
based on *Ehrlichia*  
16S rRNA gene  
sequences.**



# Discussion



- Confirmed infection of *E. chaffeensis* by PCR method in outdoor German shepherd dogs of South Korea
- To carry out regular extensive monitoring and appropriate therapy
- Control of various tick-borne disease in view of zoonosis and public health management
- Good prognosis for chemoprophylaxis and treatment to infected MWDs

# Reference

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