Original Article

J Vet Sci 2016, 17(2), 207-216 · http://dx.doi.org/10.4142/jvs.2016.17.2.207

JVS

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

Jun-Gu Kang[1,†], Sungjin Ko[1,†], W. Barney Smith[2], Heung-Chul Kim[1], In-Yong Lee[3], Joon-Seok Chae[1,*]

[1]*Laboratory of Veterinary Internal Medicine, BK21 PLUS Program for Creative Veterinary Science Research, and Research Institute for Veterinary Science, College of Veterinary Medicine, Seoul National University, Seoul 08826, Korea*

[2]*College of Veterinary Medicine, Auburn University, Auburn, AL 36849, USA*

[3]*Department of Environmental Medical Biology, Yonsei University College of Medicine, Seoul 03722, Korea*

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 *gro*EL-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

Introduction

Ticks are recognized as primary arthropod vectors of infectious disease agents that pose significant medical and veterinary health issues [7]. The incidence of tick-borne diseases (TBDs) is increasing worldwide as more pathogens are recognized [27], especially in northeastern Asian countries such as China [24,37,41], the South Korea [4,15,16,22] and Japan [35]. The transmission of zoonotic pathogens involving vertebrate hosts and ticks that interact in a constantly changing environment is often difficult to control because of zoonotic/domestic host(s), habitat distributions and transmission cycles in areas in which pastured domestic stock and wild animals coexist [32].

North Korea, which is located on the northern part of the Korean Peninsula in East Asia, is bound by China to the northeast, South Korea to the southeast, the Yellow Sea to the southwest and the Korean peninsula strait to the southeast. Tick-borne zoonotic pathogens from various sources, including wild deer [2,16,19], wild rodents [4,18], domestic animals [9,17,19] and ticks [18,30] have been reported near the southern boundary of the demilitarized zone (DMZ) separating South and North Korea. Regional prevalence and geographic and host distributions are important factors that must be understood to develop and initiate prevention strategies. Despite the geographical, political, and epidemiological importance of North Korea, screening for tick-borne pathogens has not been conducted, largely due to political and academic isolation.

In this study, tick-borne disease surveillance was conducted at a goat farm to identify tick species infesting goats and associated tick-borne zoonotic pathogens that may adversely impact veterinary and medical health in North Korea. In

Received 15 Jun. 2015, Revised 10 Sep. 2015, Accepted 7 Oct. 2015

*Corresponding author: Tel: +82-2-880-1279; Fax: +82-2-873-1213; E-mail: jschae@snu.ac.kr

†The first two authors contributed equally to this work.

pISSN 1229-845X
eISSN 1976-555X

addition, comparative phylogenetic analysis of zoonotic pathogens detected in North Korea was conducted.

Materials and Methods

Survey area and tick sampling

Blood-feeding ticks were collected from goats from April–May 2009 at farms along the coast in Rajin, Rason special economic zone, North Korea, to assess the prevalence of selected tick-borne pathogens among goats that were pastured from March–May of 1999–2009 at nearby mountainous areas. The goats grazed on indigenous shrubs, grasses, and other herbaceous vegetation, where they were exposed to questing ticks. The herd was composed of LaMancha, Erdene Black Cashmere goats from Mongolia, and Black Spanish goats from the United States. Ticks were collected from goats by placing fine tweezers placed around the mouth part, slowly removing the attached tick, and then placing up to 10 ticks in vials containing 70% ethanol. Ticks were transported to Seoul National University, where they were identified microscopically to stage of development and species according to Yamaguti *et al*. [38].

DNA preparation and polymerase chain reaction (PCR)

Identified ticks were individually homogenized mechanically using a Beadbeater TissueLyser II (Qiagen, Germany) with lysis buffer, proteinase K, and 5 mm stainless steel beads (30 frequencies/sec for 5 min), after which they were incubated at 56°C overnight and then centrifuged at 12,000 × g for 10 min at 4°C. Following centrifugation, the supernatant was used for genomic DNA extraction, which was performed with a DNeasy Tissue Kit (Qiagen) according to the manufacturer's instructions.

Table 1. Nucleotide sequences of polymerase chain reaction (PCR) primers and conditions for amplification of *Anaplasma*, *Bartonella* and *Borrelia* species genes

Species and target genes	Name of PCR primers and conditions	Primer sequences (5´- 3´)				PCR product size (bp)	References
		Denaturation (°C/min)	Annealing (°C/min)	Extension (°C/min)	Cycles		
Anaplasma and *Ehrlichia* spp. 16S rRNA	AE1-F	AAGCTTAACACATGCAAGTCGAA				1406	[28]
	AE1-R	AGTCACTGACCCAACCTTAAATG					
	Conditions	94/1	56/1	72/1.5	35		
Anaplasma (*A.*) *phagocytophilum* 16S rRNA	EE3	GTCGAACGGATTATTCTTTATAGCTTGC				926	[1]
	EE4	CCCTTCCGTTAAGAAGGATCTAATCTCC					
	Conditions	94/0.5	56/0.5	72/0.75	25		
A. bovis 16S rRNA	ABKf	TAGCTTGCTATGGGGACAA				547	[16]
	AB1r	TCTCCCGGACTCCAGTCTG					
	Conditions	94/0.5	59/0.5	72/0.5	25		
Borrelia spp. 16S rRNA	B1	CAGTGCGTCTTAAGCATGC				1427	[29]
	B8	CCTTAAATACCTTCCTCCC					
	Conditions	94/1	58/1	72/1.5	30		
	B3	GCAGCTAAGAATCTTCCGCAATGG				714	
	B6	CAACCATGCAGCACCTGTATAT					
	Conditions	94/0.5	59/0.75	72/0.75	25		
Bartonella spp. ITS	QHVE1	TTCAGATGATGATCCCAAGC				735	[20]
	QHVE3	AACATGTCTGAATATATCTTC					
	Conditions	94/0.75	55/0.75	72/0.75	30		
	QHVE12	GCAGCTAAGAATCTTCCGCAATGG				484–569	[33]
	QHVE14	CAACCATGCAGCACCTGTATAT					
	Conditions	94/0.5	58/0.75	72/0.75	30		
Bartonella spp. groEL	HspF1d	GAACTNGAAGATAAGTTNGAA				1489	[40]
	BbHS1630.n	AATCCATTCCGCCCATTC					
	Conditions	94/1.5	54/1.5	72/1.5	35		
	HSP1	GGAAAAAGTNGGCAATGAAG				888	
	HSP2	GCNGCTTCTTCACCNGCATT					
	Conditions	94/1	57/1	72/1	25		

ITS, internal transcribed spacer.

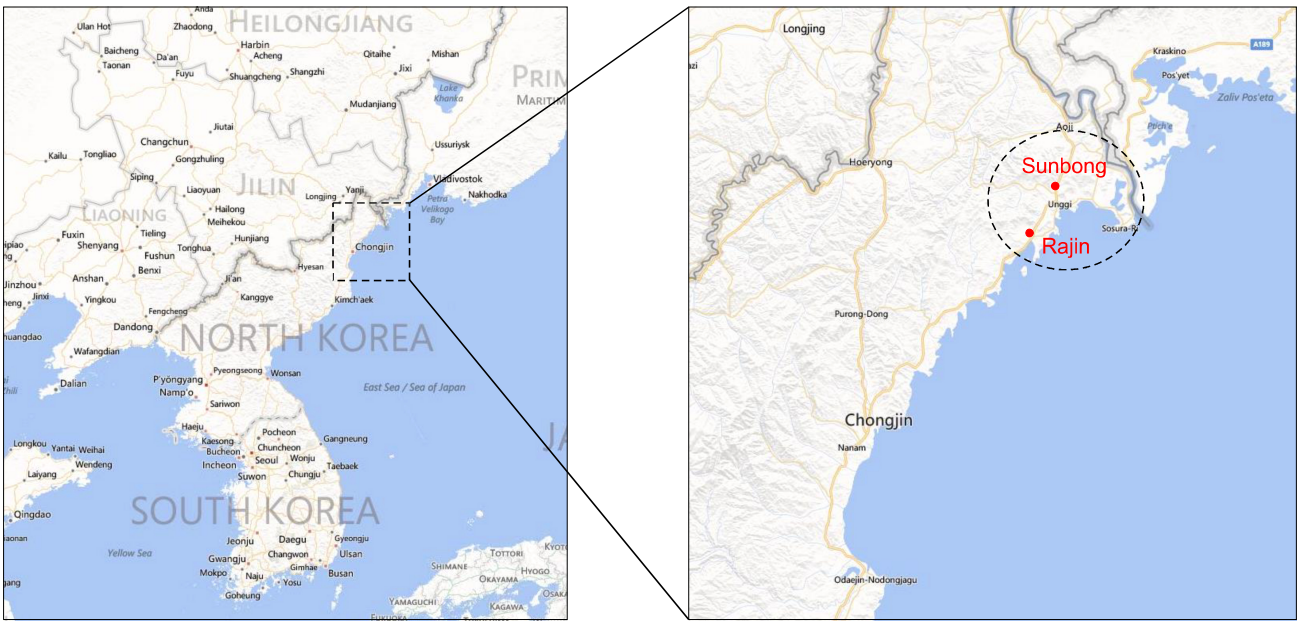

Fig. 1. Map of Rason (Rajin-Sunbong) special economic zone (black dotted circle) in North Korea. Ticks were collected from mountain-pastured goats at farms at which they were housed along the coast in Rajin.

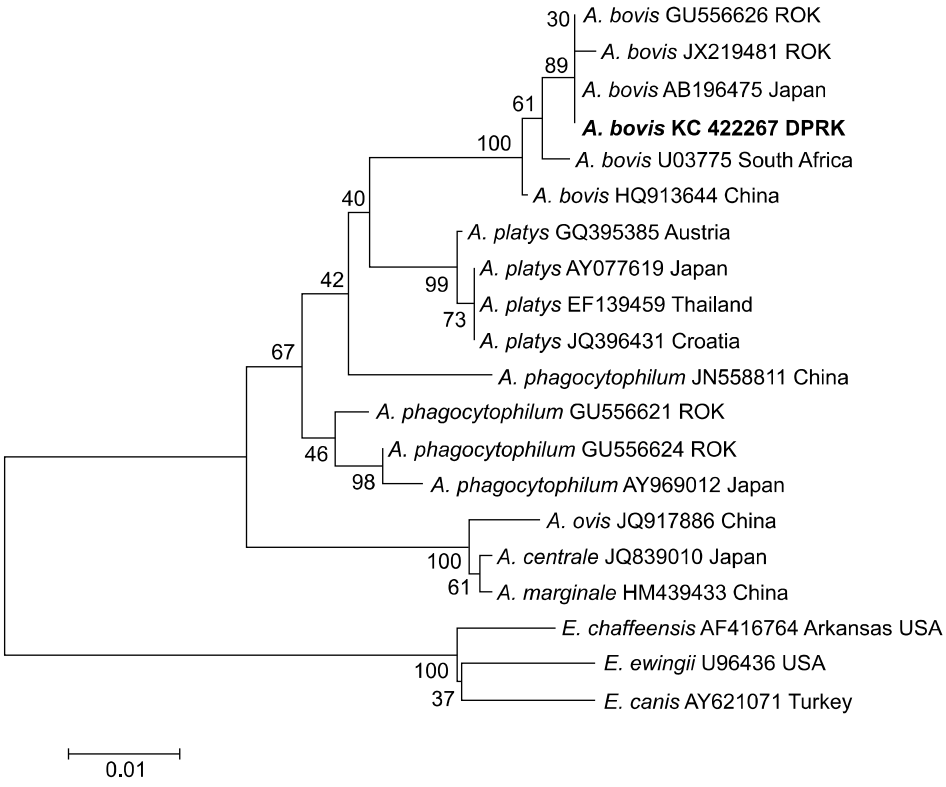

Fig. 2. Phylogenetic relationships for *Anaplsama bovis* detected from ticks in North Korea (bold letters) and *Anaplasma* and *Ehrlichia* species from other countries based on partial nucleotide sequences of the 16S rRNA gene fragment (547 bp). The neighbor-joining method was used to construct the phylogenetic tree. The numbers at the nodes are the proportions of 1,000 bootstrap iterations that support the topology shown. ROK, Republic of Korea; DPRK, Democratic People's Republic of Korea.

PCR and nested-PCR were performed using specific primer sets for *Anaplasma* (*A*.) *phagocytophilum*, *A*. *bovis*, and *Bartonella* and *Borrelia* spp. (Table 1). *A*. *phagocytophilum* genomic DNA used for the positive control was provided by J. Stephen Dumler (Johns Hopkins University School of Medicine, Baltimore, MD, USA). *Bartonella* (*B*.) *henselae* (ATCC49882) and *B*. *grahamii* (ATCC700132) were purchased from the American Type Culture Collection. PCR products were electrophoresed on 1.5% agarose gel, stained with ethidium bromide and visualized under UV light. All PCR assays were repeated a minimum of three times for confirmation.

Cloning, nucleotide sequencing and phylogenetic analysis

PCR amplicons were purified using QIAquick Gel Extraction Kits (Qiagen) and cloned using pGEM-T Easy Vectors (Promega, USA). The recombinant plasmids were transformed into *Escherichia coli* DH5α, then plated onto LB agar plates containing 100 μg/mL ampicillin. The recombinant plasmid DNA was purified using the Wizard *Plus* SV Minipreps DNA Purification System (Promega), and sequenced using a T7 and SP6 promoter primer set by dideoxy termination with an automatic sequencer (3730xl capillary DNA Analyzer; Applied Biosystems, USA). Phylogenetic analysis of the obtained sequences was conducted using Clustal X software (ver. 2.0) [21] and the neighbor- joining method with the MEGA 4.0 program [35]. Bootstrap values for the consensus tree were based on analysis of 1,000 replications.

Nucleotide sequence accession numbers

The obtained sequences in the present study were submitted to GenBank (The National Center for Biotechnology Information, USA), and accession numbers for the 16S ribosomal RNA (rRNA), *gro*EL, and internal transcribed spacer (ITS) gene sequences are shown in Figs. 2–6.

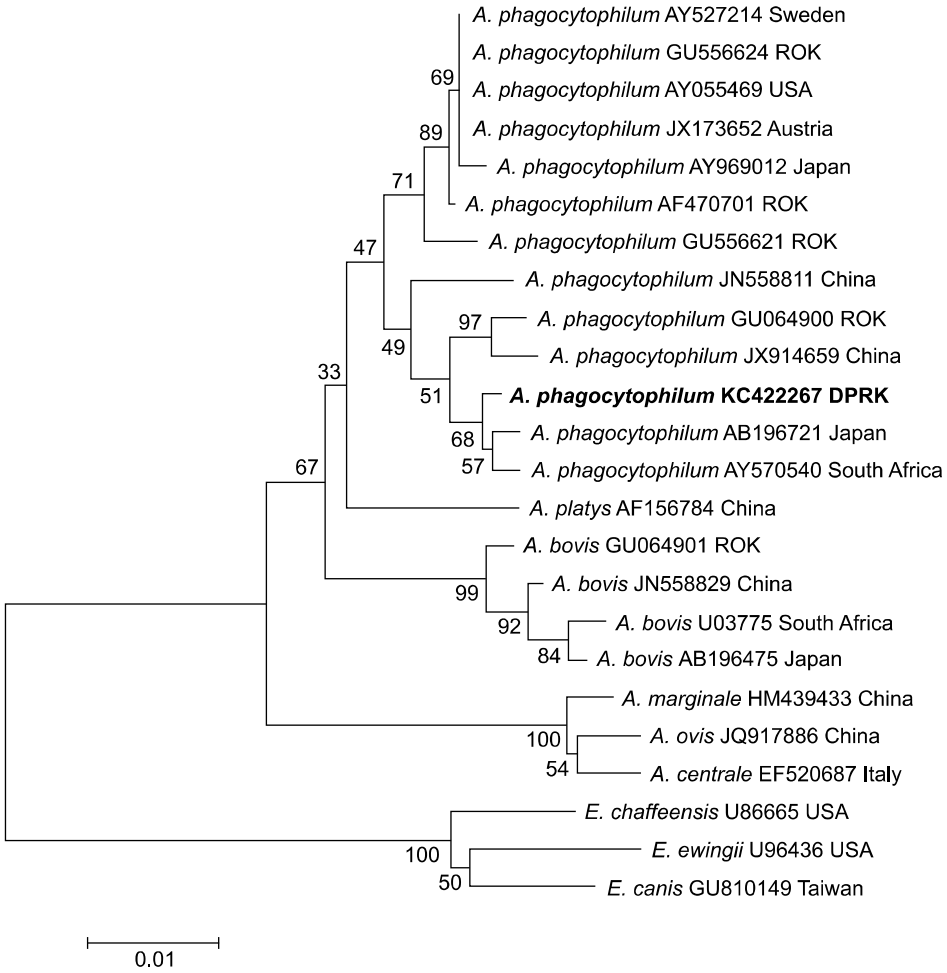

Fig. 3. Phylogenetic relationships for *Anaplsama phagocytophilum* (bold letter) detected from ticks in North Korea (bold letters) and related *Anaplasma* and *Ehrlichia* species from other countries based on partial nucleotide sequences of the 16S rRNA gene fragment (925 bp). The neighbor-joining method was used to construct the phylogenetic tree. The numbers at the nodes are the proportions of 1,000 bootstrap iterations that support the topology shown.

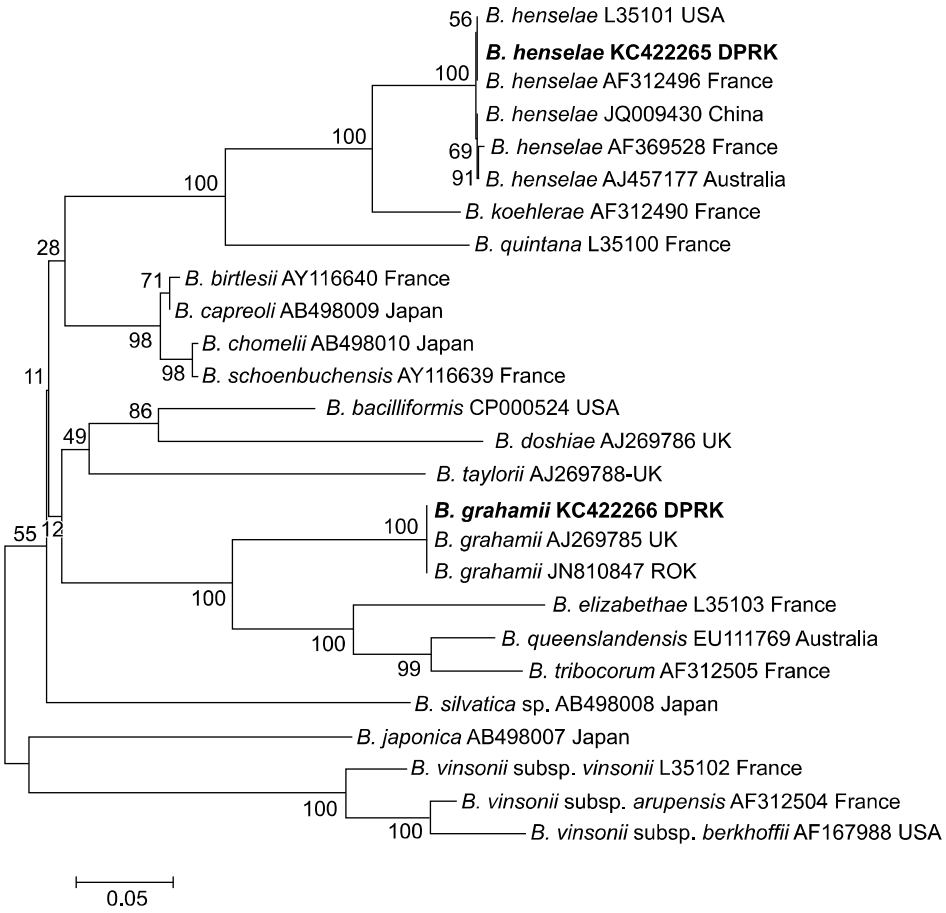

Fig. 4. Phylogenetic relationships among *Bartonella grahamii* and *Bartonella henselae* detected from ticks in North Korea (bold letters) and related *Bartonella* species from other countries based on partial nucleotide sequences of the internal transcribed spacer (ITS) gene fragment. The neighbor-joining method was used to construct the phylogenetic tree. The numbers at the nodes are the proportion of 1,000 bootstrap iterations that support the topology shown.

Results

A total of 292 (27 nymphs, 26 males, 239 females) *Haemaphysalis* (*H.*) *longicornis* Neumann were collected from domestic goats and assayed individually using generic-specific nested PCR primer sets for *Anaplasma*, *Bartonella*, *Borrelia*, and *Ehrlichia* species. A total of 77 ticks (26.4%) were positive for *A. bovis*, followed by *B. grahamii* (15, 5.1%), *A. phagocytophilum* (12, 4.1%), *B. henselae* (10, 3.4%), and *Borrelia* spp. (3, 1.0%) (Table 2). *Ehrlichia* spp. were not detected. Eleven ticks had mixed infections of two pathogens: *A. phagocytophilum* and *B. henselae* (1, 0.3%), *A. bovis* and *B. henselae* (5, 1.7%), *A. bovis* and *B. grahamii* (4, 1.4%), and *B. grahamii* and *Borrelia* spp. (1, 0.3%).

A. bovis and *A. phagocytophilum* 16S rRNA genes were detected by species-specific nested PCR. The genome sequences were analyzed and compared with partial 16S rRNA gene sequences to demonstrate genetic relationships between *Anaplasma* spp. reported from ticks elsewhere. All 77 *A. bovis* 16S rRNA gene sequences (KC422268) were identical, as were those of *A. bovis* detected in deer from South Korea (GU556626) and Japan (AB196475) (Fig. 2). All *A. phagocytophilum* 16S rRNA gene sequences (KC422267) were identical, and they demonstrated 99.6% similarity to *A. phagocytophilum* from a deer in Japan (AB196721) and a South African dog (AY570540) (Fig. 3).

Bartonella spp. ITS genes were detected by nested PCR assay. The product sizes of *B. henselae* and *B. grahamii*, including non-coding sequences, were 569 bp and 484 bp, respectively. The genome sequences were analyzed and compared with partial ITS gene sequences to demonstrate genetic relationships between *Bartonella* spp. All 12 *B. henselae* ITS gene sequences from North Korea (KC422265) were identical and were also identical to *B. henselae* sequences detected in humans from France (AF312496) and the United States (L35101) (Fig. 4). Moreover, *B. grahamii* ITS gene sequences (KC422266) from North Korea were identical, as were those of *B. grahamii* from a mouse from the UK

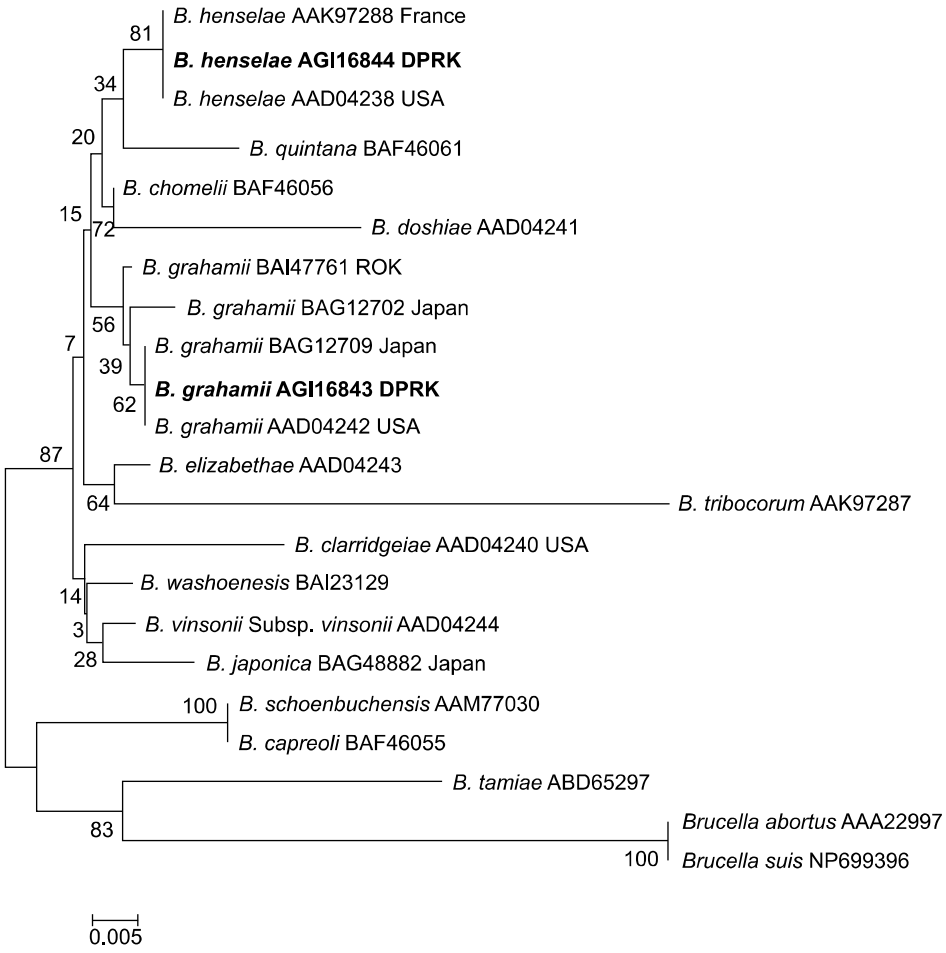

Fig. 5. Phylogenetic relationships for *Bartonella grahamii* and *Bartonella henselae* detected from ticks in North Korea (bold letters) and *Bartonella* species from other countries based on partial protein sequences of the 409 amino acid *gro*EL gene fragment. The neighbor-joining method was used to construct the phylogenetic tree. The numbers at the nodes are the proportion of 1,000 bootstrap iterations that support the topology shown.

(AJ269785) and a South Korean deer (JN810847) (Fig. 4).

*gro*EL specific nested PCR revealed that the product size of six *B. grahamii* (KC422270) and one *B. henselae* (KC422271) *gro*EL gene was 888 bp and that the gene encoded 295 amino acids of *gro*EL. The six *B. grahamii* sequences of the *gro*EL amino acids were identical and corresponded to *B. grahamii* mice from the UK (BAG12709) and Japan (AAD04238) (Fig. 5). One *B. henselae* sequence of the *gro*EL amino acids corresponded to *B. henselae* from the United States (AAD04238) and France (AAK97288).

A total of three ticks were positive for *Borrelia* spp. using the 16S rRNA genes, and these were identical and demonstrated 99.0% homology to *B. theileri* (NR025874) (Fig. 6).

Discussion

Little information is available regarding the causative agents of tick-borne diseases of veterinary and medical importance in North Korea, in part because of strict access limitations [41]. Preliminary data based on a high prevalence of known and potential (not identified to species) pathogens observed in *H. longicornis* collected from goats that were pastured in the mountainous terrain in North Korea illustrate the need for further studies investigating the impact of tick-borne diseases among domestic animals, as well as humans.

In South Korea, *H. longicornis* is the tick most commonly collected from grasses and herbaceous vegetation habitats, and these have been shown to have high tick-borne infection rates [4,18,28]. *A. bovis*, the causative agent of benign bovine rickettsiosis, is widely found in ticks collected from cattle and surrounding vegetation throughout much of Africa and Asia [9,11,26]. In South Korea, *A. bovis* has been identified in *H. longicornis* collected from deer, birds, cattle, and vegetation (tick drag) [15-17,22,28]. In Japan, *A. bovis* infection rates among sika deer and co-pastured cattle were 23% and 15%, respectively [14]. While *A. bovis* is a pathogen of veterinary

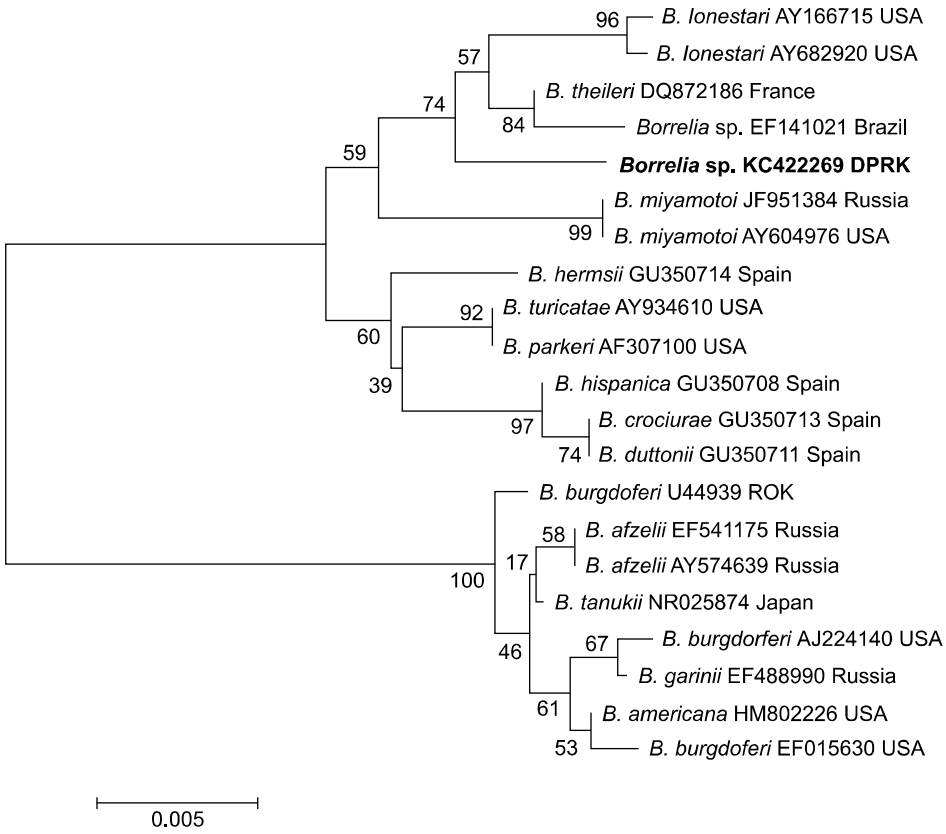

Fig. 6. Phylogenetic relationships among *Borrelia* spp. detected from ticks in North Korea (bold letters) and related *Borrelia* spp. from other countries based on partial nucleotide sequences of the 16S rRNA gene fragment (705 bp). The neighbor-joining method was used to construct the phylogenetic tree. The numbers at the nodes are the proportion of 1,000 bootstrap iterations that support the topology shown.

Table 2. Prevalence of *Anaplasma* (*A.*) *phagocytophilum*, *A. bovis*, *Bartonella* spp., and *Borrelia* spp. targeting the 16S rRNA gene fragment from *Haemaphysalis longicornis* ticks collected from goats in North Korea

Stages	Number of ticks	Number of amplicon sequences (%)								
		A. bovis	*A. phagocytophilum*	*Bartonella* (*B.*) *henselae*	*B. grahamii*	*Borrelia* spp.	*A. phagocytophilum* and *B. henselae**	*A. bovis* and *B. henselae**	*A. bovis* and *B. grahamii**	*B. grahamii* and *Borrelia* spp.*
Nymph	27	2 (7.4)	0	0	0	0	0	0	0	0
Male	26	4 (15.4)	0	0	0	0	0	0	0	0
Female	239	71 (29.7)	12 (5.0)	10 (4.2)	15 (6.3)	3 (1.3)	1 (0.4)	5 (2.1)	4 (1.7)	1 (0.4)
Total	292	77 (26.4)	12 (4.1)	10 (3.4)	15 (5.1)	3 (1.0)	1 (0.3)	5 (1.7)	4 (1.4)	1 (0.3)

*Dual infection.

importance, it is not known to cause human disease [31]. *A. phagocytophilum*, the causative agent of human granulocytic anaplasmosis, was detected in *H. longicornis* collected from goats in North Korea and has also been detected from *H. longicornis* and wild deer in South Korea [10,16,22,28] and from goats, cattle, dogs, and ticks collected from vegetation in China [41]. Our survey showed high infection rates of *A. bovis* (27.3%) among *H. longicornis* collected from goats in North Korea, indicating that they are of veterinary concern, while *A. phagocytophilum* (4.3%) poses a medical threat to goat herders and others exposed to questing ticks in North Korea. Both *A. bovis* and *A. phagocytophilum* showed a high degree of

homology to species from neighboring countries, indicating that they have widespread distribution throughout eastern Asia.

Evidence suggests that many *Bartonella* spp. (*e.g.*, *B. henselae*, *B. vinsonii*, *B. bovis*, *B. elizabethae*, *B. washoensis*, and *B. clarridgeiae*) are the causative agents of various diseases in people and/or animals [5]. While the cat flea (*Ctenocephalides felis*) and rodent flea (*Ctenocephalides nobilis*) have been identified as the primary vectors of *B. henselae* and *B. grahamii*, respectively [5,12], *H. longicornis* collected from goats in North Korea demonstrated high infection rates. *Bartonella* spp. are distributed worldwide and transmitted by a wide variety of vectors, including ticks, flies, body lice, mites, and sandflies [5,36]. More recently, various species of ticks have been shown to be positive for *Bartonella* spp. by PCR [3], and *B. henselae* has been shown to be transmitted through the salivary contents of *Ixodes* spp. [8]. In a previous study, *Bartonella* DNA was detected by PCR in *H. flava*, *H. longicornis*, *I. nipponensis*, and *I. turdus* ticks collected from vegetation (tick drag) and/or from rodents captured in rural areas and US military training sites in South Korea [18]. In the present study, *B. grahamii* and *B. henselae* ITS gene fragments were detected and DNA sequences corresponded to *B. grahamii* and *B. henselae* ITS gene sequences in GenBank. Since there were insufficient ITS gene sequences available in GenBank for a comprehensive comparison, additional phylogenetic analysis using the *gro*EL gene was conducted by nested PCR. A total of 7/25 (28%) of the *gro*EL gene sequences (1 *B. henselae* and 6 *B. grahamii*) were positive, and their translocated amino acids sequences corresponded to *B. grahamii* and *B. henselae* sequences reported from other countries in GenBank, respectively. Thus, molecular evidence implicates ticks as potential vectors of *Bartonella* species in Korea.

Borrelia species are transmitted by *Ixodid* ticks, and have recently been detected by PCR from *H. longicornis* in China, *H. flava* in Japan, and from *I. persulcatus*, *I. nipponensis*, and *I. turdus* in South Korea [4,13,15,30,34]. *H. longicornis* in North Korea were found to be positive for *Borrelia* spp. by PCR using 16S rRNA primer sets and gene sequences compared with those available in GenBank. Prior to this survey, *Borrelia* spp. had not been detected from *Haemaphysalis* spp. and *I. nipponensis* collected from the Korean Peninsula [4,23,30]. While Lyme borreliosis was not a reportable disease in South Korea until 2010, from 2005−2012, there were 11 autochthonous cases reported, mostly in the northern part of South Korea, which were suspected to be due to the greater abundance of *I. persulcatus* [25]. However, based on the results of the present study, *H. longicornis*, the predominant tick species in grasslands, pastures, and gravesites in forested areas in South Korea [6], is a potential vector of *Borrelia* species.

In conclusion, various species of ticks are known vectors or implicated as vectors *Anaplasma*, *Borrelia*, *Bartonella*, and *Ehrlichia* spp. A rapidly expanding number of reservoir-adapted pathogens have been discovered among a wide range of species of ticks as known or suspected vectors of zoonotic tick-borne pathogens. The results of the present study implicates *H. longicornis* as a potential vector of *Anaplasma*, *Bartonella*, and *Borrelia* spp. in the North Korea and indicates that it will impact veterinary and medical health in North Korea.

Acknowledgments

This research was partially supported by a National Research Foundation of Korea Grant funded by the Korean Government (2014R1A1A2056207) and was partially funded by the BK21 PLUS Program for Creative Veterinary Science Research, Research Institute for Veterinary Science and College of Veterinary Medicine, Seoul National University in Korea.

Conflict of Interest

There is no conflict of interest.

References

1. **Barlough JE, Madigan JE, Derock E, Bigornia L.** Nested polymerase chain reaction for detection of *Ehrlichia equi* genomic DNA in horses and ticks (*Ixodes pacificus*). Vet Parasitol 1996, **63**, 319-329.
2. **Biesiada G, Czepiel J, Leśniak MR, Garlicki A, Mach T.** Lyme disease: review. Arch Med Sci 2012, **8**, 978-982.
3. **Billeter SA, Levy MG, Chomel BB, Breitschwerdt EB.** Vector transmission of *Bartonella* species with emphasis on the potential for tick transmission. Med Vet Entomol 2008, **22**, 1-15.
4. **Chae JS, Yu DH, Shringi S, Klein TA, Kim HC, Chong ST, Lee IY, Foley J.** Microbial pathogens in ticks, rodents and a shrew in northern Gyeonggi-do near the DMZ, Korea. J Vet Sci 2008, **9**, 285-293.
5. **Chomel BB, Boulouis HJ, Maruyama S, Breitschwerdt EB.** *Bartonella* spp. in pets and effect on human health. Emerg Infect Dis 2006, **12**, 389-394.
6. **Chong ST, Kim HC, Lee IY, Kollars TM Jr, Sancho AR, Sames WJ, Chae JS, Klein TA.** Seasonal distribution of ticks in four habitats near the demilitarized zone, Gyeonggi-do (province), Republic of Korea. Korean J Parasitol 2013, **51**, 319-325.
7. **Colwell DD, Dantas-Torres F, Otranto D.** Vector-borne parasitic zoonoses: emerging scenarios and new perspectives. Vet Parasitol 2011, **182**, 14-21.
8. **Cotté V, Bonnet S, Le Rhun D, Le Naour E, Chauvin A, Boulouis HJ, Lecuelle B, Lilin T, Vayssier-Taussat M.** Transmission of *Bartonella henselae* by *Ixodes ricinus*. Emerg Infect Dis 2008, **14**, 1074-1080.
9. **Doan HTT, Hoh JH, Choe SE, Yoo MS, Kim YH, Reddy KE, Quyen DV, Nguyen LTK, Nguyen TTD, Kweon CH, Jung SC, Chang KY, Kang SW.** Molecular detection and phylogenetic analysis of *Anaplasma bovis* from *Haemaphysalis longicornis* feeding on grazing cattle in Korea. Vet Parasitol

2013, **196**, 478-481.
10. **Dumler JS.** The biological basis of severe outcomes in *Anaplasma phagocytophilum* infection. FEMS Immunol Med Microbiol 2012, **64**, 13-20.
11. **Harrus S, Perlman-Avrahami A, Mumcuoglu KY, Morick D, Eyal O, Baneth G.** Molecular detection of *Ehrlichia canis*, *Anaplasma bovis*, *Anaplasma platys*, *Candidatus* Midichloria mitochondrii, and *Babesia canis* vogeli in ticks from Israel. Clin Microbiol Infect 2011, **17**, 459-463.
12. **Higgins JA, Radulovic S, Jaworski DC, Azad AF.** Acquisition of the cat scratch disease agent *Bartonella henselae* by cat fleas (Siphonaptera: Pulicidae). J Med Entomol 1996, **33**, 490-495.
13. **Ishiguro F, Takada N, Masuzawa T, Fukui T.** Prevalence of Lyme disease *Borrelia* spp. in ticks from migratory birds on the Japanese mainland. Appl Environ Microbiol 2000, **66**, 982-986.
14. **Jilintai, Seino N, Hayakawa D, Suzuki M, Hata H, Kondo S, Matsumoto K, Yokoyama N, Inokuma H.** Molecular survey of *Anaplasma bovis* and *Anaplasma phagocytophilum* infection in cattle in a pastureland where sika deer appear in Hokkaido, Japan. Jpn J Infect Dis 2009, **62**, 73-75.
15. **Kang JG, Kim HC, Choi CY, Nam HY, Chae HY, Chong ST, Klein TA, Ko S, Chae JS.** Molecular detection of *Anaplasma*, *Bartonella*, and *Borrelia* species in ticks collected from migratory birds from Hong-do Island, Republic of Korea. Vector Borne Zoonotic Dis 2013, **13**, 215-225.
16. **Kang JG, Ko S, Kim YJ, Yang HJ, Lee H, Shin NS, Choi KS, Chae JS.** New genetic variants of *Anaplasma phagocytophilum* and *Anaplasma bovis* from Korean water deer (*Hydropotes inermis argyropus*). Vector Borne Zoonotic Dis 2011, **11**, 929-938.
17. **Kang SW, Doan HTT, Choe SE, Noh JH, Yoo MS, Reddy KE, Kim YH, Kweon CH, Jung SC, Chang KY.** Molecular investigation of tick-borne pathogens in ticks from grazing cattle in Korea. Parasitol Int 2013, **62**, 276-282.
18. **Kim CM, Yi YH, Yu DH, Lee MJ, Cho MR, Desai AR, Shringi S, Klein TA, Kim HC, Song JW, Baek LJ, Chong ST, O'Guinn ML, Lee JS, Lee IY, Park JH, Foley J, Chae JS.** Tick-borne rickettsial pathogens in ticks and small mammals in Korea. Appl Environ Microbiol 2006, **72**, 5766-5776.
19. **Ko S, Kim SJ, Kang JG, Won S, Lee H, Shin NS, Choi KS, Youn HY, Chae JS.** Molecular detection of *Bartonella grahamii* and *B. schoenbuchensis*-related species in Korean water deer (*Hydropotes inermis argyropus*). Vector Borne Zoonotic Dis 2013, **13**, 415-418.
20. **La Scola B, Raoult D.** Culture of *Bartonella quintana* and *Bartonella henselae* from human samples: a 5-year experience (1993 to 1998). J Clin Microbiol 1999, **37**, 1899-1095.
21. **Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG.** Clustal W and Clustal X version 2.0. Bioinformatics 2007, **23**, 2947-2948.
22. **Lee MJ, Chae JS.** Molecular detection of *Ehrlichia chaffeensis* and *Anaplasma bovis* in the salivary glands from *Haemaphysalis longicornis* ticks. Vector Borne Zoonotic Dis 2010, **10**, 411-413.
23. **Lee SH, Kim BJ, Kim JH, Park KH, Yeo SJ, Kim SJ, Kook YH.** Characterization of *Borrelia burgdorferi* strains isolated from Korea by 16S rDNA sequence analysis and PCR-RFLP analysis of *rrf* (5S)-*rrl* (23S) intergenic spacer amplicons. Int J Sys Evol Microbiol 2000, **50**, 857-863.
24. **Liu S, Yuan C, Cui YF, Li BX, Wu LJ, Liu Y.** Investigation of *Borrelia* spp. in ticks (Acari: Ixodidae) at the border crossings between China and Russia in Heilongjiang Province, China. Asian Pac J Trop Med 2012, **5**, 459-464.
25. **Moon S, Hong Y, Hwang KJ, Kim S, Eom J, Kwon D, Park JH, Youn SK, Sohn A.** Epidemiological features and clinical manifestations of Lyme borreliosis in Korea during the period 2005-2012. Jpn J Infect Dis 2015, **68**, 1-4.
26. **Nair AS, Ravindran R, Lakshmanan B, Sreekumar C, Kumar SS, Raju R, Tresamol PV, Vimalkumar MB, Saseendranath MR.** Bovine carriers of *Anaplasma marginale* and *Anaplasma bovis* in South India. Trop Biomed 2013, **30**, 105-112.
27. **Nicholson WL, Allen KE, McQuiston JH, Breitschwerdt EB, Little SE.** The increasing recognition of rickettsial pathogens in dogs and people. Trends Parasitol 2010, **26**, 205-212.
28. **Oh JY, Moon BC, Bae BK, Shin EH, Ko YH, Kim YJ, Park YH, Chae JS.** Genetic identification and phylogenetic analysis of *Anaplasma* and *Ehrlichia* species in *Haemaphysalis longicornis* collected from Jeju island, Korea. J Bacteriol Virol 2009, **39**, 257-267.
29. **Park HS, Lee JH, Jeong EJ, Koh SE, Park TK, Jang WJ, Park KH, Kim BJ, Kook YH, Lee SH.** Evaluation of *groEL* gene analysis for identification of *Borrelia burgdorferi* sensu lato. J Clin Microbiol 2004, **42**, 1270-1273.
30. **Park KH, Lee SH, Won WJ, Jang WJ, Chang WH.** Isolation of *Borrelia burgdorferi*, the causative agent of Lyme disease, from *Ixodes* ticks in Korea. J Korean Soc Microbiol 1992, **27**, 307-312.
31. **Rymaszewska A, Grenda S.** Bacteria of the genus *Anaplasma* – characteristics of *Anaplasma* and their vectors: a review. Vet Med (Praha) 2008, **53**, 573-584.
32. **Salkeld DJ, Lane RS.** Community ecology and disease risk: lizards, squirrels, and the Lyme disease spirochete in California, USA. Ecology. 2010, **91**, 293-8.
33. **Seki N, Sasaki T, Sawabe K, Sasaki T, Matsuoka M, Arakawa Y, Marui E, Kobayashi M.** Epidemiological studies on *Bartonella quintana* infections among homeless people in Tokyo, Japan. Jpn J Infect Dis 2006, **59**, 31-35.
34. **Sun J, Liu Q, Lu L, Ding G, Guo J, Fu G, Zhang J, Meng F, Wu H, Song X, Ren D, Li D, Guo Y, Wang J, Li G, Liu J, Lin H.** Coinfection with four genera of bacteria (*Borrelia*, *Bartonella*, *Anaplasma*, and *Ehrlichia*) in *Haemaphysalis longicornis* and *Ixodes sinensis* ticks from China. Vector Borne Zoonotic Dis 2008, **8**, 791-795.
35. **Tamura K, Dudley J, Nei M, Kumar S.** MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol 2007, **24**, 1596-1599.
36. **Tsai YL, Chang CC, Chuang ST, Chomel BB.** *Bartonella* species and their ectoparasites: selective host adaptation or strain selection between the vector and the mammalian host? Comp Immunol Microbiol Infect Dis 2011, **34**, 299-314.

37. **Xia Z, Yu D, Mao J, Zhang Z, Yu J.** The occurrence of *Dirofilaria immitis*, *Borrelia burgdorferi*, *Ehrlichia canis* and *Anaplasma phagocytophium* in dogs in China. J Helminthol 2012, **86**, 185-189.
38. **Yamaguti N, Tipton VJ, Keegan HL, Toshioka S.** Ticks of Japan, Korea and the Ryukyu Islands. Sci Bull Biol Ser 1971, **15**, 1-227.
39. **Yoshii K, Mottate K, Omori-Urabe Y, Chiba Y, Seto T, Sanada T, Maeda J, Obara M, Ando S, Ito N, Sugiyama M, Sato H, Fukushima H, Kariwa H, Takashima I.** Epizootiological study of tick-borne encephalitis virus infection in Japan. J Vet Med Sci 2011, **73**, 409-412.
40. **Zeaiter Z, Fournier PE, Ogata H, Raoult D.** Phylogenetic classification of *Bartonella* species by comparing *groEL* sequences. Int J Syst Evol Microbiol 2002, **52**, 165-171.
41. **Zhang L, Liu H, Xu B, Lu Q, Li L, Chang L, Zhang X, Fan D, Li G, Jin Y, Cui F, Shi Y, Li W, Xu J, Yu XJ.** *Anaplasma phagocytophilum* infection in domestic animals in ten provinces/cities of China. Am J Trop Med Hyg 2012, **87**, 185-189.