Molecular Investigation of Tick-Borne Pathogens in Ticks removed from Tick-Bitten Humans in the Republic of Korea

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Research

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Abstract

Background Tick-borne infections are continuously increasing due to climate change, increased outdoor activities and increased travel between countries. This study was to investigate the presence of tick-borne pathogens in ticks removed from tick-bitten humans in southwest provinces of Republic of Korea (ROK).

Methods Ticks were obtained from those tick-bitten humans between May 2014 and September 2017 in Jeollanam provinces and Gwangju metropolitan city in ROK. The presence of the tick-borne pathogens in ticks removed from tick-bitten humans was analyzed using pathogen-specific polymerase chain reaction (PCR).

Results We identified 33 ticks from three tick species, namely Amblyomma testudinarium (60.6%), Haemaphysalis longicornis (27.3%), and Ixodes nipponensis (12.1%) in order of occurrence by morphology and 16S rDNA-targeting PCR. Tick-borne pathogens were found in 16 ticks using pathogen-specific PCR. From the results, 12 ticks (36.4%) tested positive for spotted fever group (SFG) Rickettsia: Rickettsia monacensis (1/12), R. tamurae (8/12), and Candidatus Rickettsia jingxinensis (3/12). Three ticks (9.1%) were positive for Anaplasma phagocytophilum . In addition, three ticks (9.1%) tested positive for Babesia gibsoni (1/3) and B. microti (2/3).

Conclusions In conclusion, we identified three tick species; the most common species was A. testudinarium followed by H. longicornis and I. nipponensis . SFG Rickettsia , A. phagocytophilum , and Babesia spp. were the most frequently detected pathogens in ticks removed from tick-bitten humans. R. tamurae and Ca. R. jingxinensis were firstly detected in Korea. The present results will contribute to the understanding of tick-borne infections in animals and humans in the ROK.

Background

Ticks are major vectors of pathogens such as bacteria, viruses, and protozoans. These arthropods can transmit a variety of diseases to humans and animals (1). Tick-borne diseases are caused by viral or bacterial pathogens transmitted through tick bites. Several tick-borne diseases such as Lyme disease (caused by Borrelia species), spotted fever group rickettsioses (caused by Rickettsia spp.), anaplasmosis (caused by Anaplasma phagocytophilum), bartonellosis (caused by Bartonella spp.), Q fever (caused by Coxiella burnetii), and babesiosis (caused by Babesia spp.) have been reported in the Republic of Korea (ROK) (2).

The incidence of tick-borne diseases in the ROK is increasing due to global warming, increased outdoor activities, and increased international travel. The growing number of tick bites each year poses an escalating risk of tick-borne diseases (2). Few studies have investigated the prevalence of tick-borne pathogens in ticks removed from tick-bitten humans in the ROK. However, it is necessary to determine the extent of tick-borne pathogens in the ROK, and to characterize them.

The present study aimed to investigate the presence of tick-borne pathogens in ticks removed from humans in the southwest provinces of the ROK. Our study detected the DNA of tick-borne pathogens from ticks using pathogen-specific nested PCR. The results of this study will contribute to the understanding of the interaction between ticks and pathogens that cause diseases in humans.

Methods

Tick samples

Ticks were removed from humans between May 2014 and September 2017 in the Jeollanam Provinces and Gwangju Metropolitan City in the ROK. All ticks were morphologically identified according to species and life stage using a microscope and standard taxonomic keys. Ticks were washed in 70% ethanol, rinsed twice with sterile phosphate-buffered saline (PBS), added to a hard tissue grinding MK28 tube (Bertin Technology, Rockville, MD, USA) containing 800 µL of PBS with 1x PC/SM (penicillin and streptomycin), ground using a FastPrep®-24 Classic instrument (MP Biomedicals, Solon, OH, USA), and stored at -80 °C until used for DNA extraction.

DNA extraction

We mixed 150 µL of the ground tick with 150 µL buffer ATL and 20 µL proteinase K, and incubated at 56 °C overnight for lysis; the genomic DNA was extracted using a QIAamp Tissue & Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA was eluted into 50 µL TE buffer and stored at -20 °C until PCR amplification.

Polymerase Chain Reaction (PCR)

To detect the presence of Rickettsia DNA, the outer membrane protein A gene (ompA), citrate synthase gene (gltA), and a 17 kDa protein gene (17 kDa) of the spotted fever group Rickettsia species were targeted. The heat shock protein gene (groEL) and the ankyrin-related protein gene (ankA) were targeted to detect A. phagocytophilum. To detect the presence of Borrelia DNA, the CTP synthase gene (pyrG) was targeted. The 16S-23S internal transcribed spacer region (ITS) was targeted to detect Bartonella species. The htpAB-associated repetitive element (IS1111) was targeted to detect Coxiella species. To detect the presence of Babesia species, 18S rDNA was targeted. To identify tick species, conventional PCR targeting the mitochondrial 16S rRNA gene (16S rDNA) was performed. All PCR primers used for detecting tick-borne pathogens, PCR conditions, and product sizes are given in Table 1. Conventional PCR (C-PCR) was performed in 20 µL reaction volumes using the AccuPowerR PCR PreMix (Bioneer Corp., Korea). Each PCR mixture consisted of 16 µL of distilled water, 1 µL of each primer (10 pmol/µL), and 2 µL of genomic DNA as template DNA. For 16S rDNA C-PCR and 18S rDNA nested PCR (N-PCR), we performed PCR using AmpliTaq Gold 360 Master Mix (Applied Biosystems, CA, USA) instead of AccuPowerR PCR PreMix.

Table 1

Oligonucleotide primers and PCR conditions used for the detection of tick-borne pathogens in ticks removed from tick-bitten humans.

Identification	Target gene[a]	Primer name	Nucleotide sequence (5'-3')	Product size (bp)	PCR conditions (°C/sec)				Refere
					Denaturation	Annealing	Extension	Cycles	
Rickettsia species	ompA	RR190.70F	ATGGCGAATATTTCTCCAAAAA	634	94/30	50/30	72/60	40	(3, 4)
		RR190.701R	GTTCCGTTAATGGCAGCATCT						
		RR190.70F	ATGGCGAATATTTCTCCAAAAA	535	94/30	50/30	72/30	5	(3)
		RR190.602R	AGTGCAGCATTCGCTCCCCCT		94/30	54/30	72/30	30	
	gltA	GLTA1F	GACGGTGATAAAGGAATCTTG	1022	95/20	47/30	72/60	40	(5)
		GLTA1R	CATTTCTTTCCATTGTGCCATC						
		GLTA2F	CTACGAACTTACCGCTATTAG	446	95/20	43/30	72/30	5	
		GLTA2R	GACCAAAACCCATTAACCTAAAC		95/20	48/30	72/30	30	
	17 kDa	Rr17k.1p	TTTACAAAATTCTAAAAACCAT	539	95/30	57/60	72/120	35	(6)
		Rr17k.539n	TCAATTCACAACTTGCCATT						
		Rr17k.90p	GCTCTTGCAACTTCTATGTT	450	95/30	57/60	72/120	35	
		Rr17k.539n	TCAATTCACAACTTGCCATT						
Anaplasma phagocytophilum	groEL	GRO607F	GAAGATGCWGTWGGWTGTACKGC	688	95/30	54/30	72/60	30	(7)
		GRO1294R	AGMGCTTCWCCTTCWACRTCYTC						
		GRO677F	ATTACTCAGAGTGCTTCTCARTG	445	95/30	57/30	72/60	30	
		GRO1121R	TGCATACCRTCAGTYTTTTCAAC						
	ankA	ANK-F1	GAAGAAATTACAACTCCTGAAG	705	95/30	53/30	72/60	35	(8)
		ANK-R1	CAGCCAGATGCAGTAACGTG						
		ANK-F2	TTGACCGCTGAAGCACTAAC	664	95/30	52/30	72/60	5	
		ANK-R2	ACCATTTGCTTCTTGAGGAG		95/30	54/30	72/60	25	
Borrelia species	pyrG	pyrG-1F	ATTGCAAGTTCTGAGAATA	801	94/20	45/30	72/30	30	(9)
		pyrG-1R	CAAACATTACGAGCAAATTC						
		pyrG-2F	GATATGGAAAATATTTTATTTATTG	707	95/30	45/30	72/30	5	
					95/30	47/30	72/30	5	
		pyrG-2R	AAACCAAGACAAATTCCAAG		95/30	49/30	72/30	25	
Bartonella species	ITS	ITS_OF	TTCAGATGATGATCCCAAGC	639	95/30	48/30	72/60	5	(10)
		ITS_OR	AACATGTCTGAATATATCTTC		95/30	50/30	72/60	30	
		ITS_IF	CCGGAGGGCTTGTAGCTCAG	499	95/30	41/30	72/60	30	
		ITS_IR	CACAATTTCAATAGAAC						
Coxiella burnetii	IS1111	IS111F1	TACTGGGTGTTGATATTGC	485	95/15	52/5	72/30	35	(11)
		IS111R1	CCGTTTCATCCGCGGTG						
		IS111F2	GTAAAGTGATCTACACGA	260	95/15	56/15	72/15	30	
		IS111R2	TTAACAGCGCTTGAACGT						
Babesia species	18S rDNA	Bab5	AATTACCCAATCCTGACACAGG	485	94/60	55/60	72/120	35	(12)
		Bab8	TTTGGCAGTAGTTCGTCTTTAACA						
		Bab6	GACACAGGGGGTAGTGACAAGA	407	94/60	55/60	72/120	30	
		Bab7	CCCAACTGCTCCTATTAACCATTAC						
Ticks	16S rDNA	16S + 1-F	CTGCTCAATGAATATTTAAATTGC	450	95/45	55/60	72/90	40	(13)

[a] ompA, outer membrane protein A gene; gltA, citrate synthase gene; 17 kDa, 17 kDa protein gene; groEL, heat shock protein gene; ankA, ankyrin-related protei gene; pyrG, CTP synthase gene; ITS, 16S-23S internal transcribed spacer region; IS1111, htpAB-associated repetitive element; 18S rDNA, 18S ribosomal RNA gene; 16S rDNA, 16S ribosomal RNA gene

	16S + 1-R	CGGTCTAAACTCAGATCATGTAGG

[a] ompA, outer membrane protein A gene; gltA, citrate synthase gene; 17 kDa, 17 kDa protein gene; groEL, heat shock protein gene; ankA, ankyrin-related protei gene; pyrG, CTP synthase gene; ITS, 16S-23S internal transcribed spacer region; IS1111, htpAB-associated repetitive element; 18S rDNA, 18S ribosomal RNA gene; 16S rDNA, 16S ribosomal RNA gene

For N-PCR, the reaction mixture was identical to that used in C-PCR, except that the first PCR product was used as template DNA, and the N-PCR primers were included. With each PCR run, a positive and a negative control (molecular grade water) were included.

All amplifications were performed in an AB thermal cycler (Applied Biosystem, Foster City, CA, USA). The amplified products were separated by electrophoresis on a 1.2% agarose gel, and stained with ethidium bromide for visualization.

Sequencing and phylogenetic analysis

The amplified PCR products were purified using QIAquick PCR purification kits (QIAGEN, Hilden, Germany) and sequenced with the PCR primers at Solgent Inc. (Daejeon, Korea). The sequences obtained in this study were compared for similarity with the GenBank sequences using BLAST. Gene sequences, excluding the primer regions, were aligned using the multisequence alignment program in Lasergene version 8 (DNASTAR, USA).

Phylogenetic trees were constructed using ClustalW of the MegAlign Program (DNASTAR, USA) based on the alignments of positive gene sequences using the neighbor-joining method. Bootstrap analysis (1,000 replicates) was performed according to the Kimura 2-parameter method. Pairwise alignments were performed with an open-gap penalty of 10 and a gap extension penalty of 0.5.

Results

Tick identification

We obtained 33 ticks from 30 tick-bitten humans. Out of these, 15 ticks (45.5%) were adults, namely 12 females and 3 males, and 18 ticks (54.5%) were nymphs. Based on morphological examination using a microscope for tick identification, the ticks were identified as Amblyomma testudinarium (20, 60.6%; 7 adults and 13 nymphs), Haemaphysalis longicornis (9, 27.3%; 5 adults and 4 nymphs), and Ixodes nipponensis (4, 12.1%; 3 adults and 1 nymph), as described in Table 2. Tick identification using 16S rDNA C-PCR and DNA sequencing yielded the same results as the microscopic examination with the exception of four samples without tick DNA (shown in Table 4).

Table 2
Developmental stages and species of ticks removed from tick-bitten humans determined by both morphological identification and 16S rDNA-targeting conventional PCR

Tick species		Amblyomma testudinarium	Heamaphysalis longicornis	Ixodes nipponensis
Development stage	Adult female	4	5	3
	Adult male	3	0	0
	Nymph	13	4	1
	Larva	0	0	0
	Total No. (%)	20 (60.6%)	9 (27.3%)	4 (12.1%)
		33 (100%)		

Table 4

Characteristics of 33 ticks using the DNA of tick-borne pathogens obtained from 30 tick-bitten humans

Patient no.	Patient age/sex	Tick species identified by a microscopy	Development stage (sex)	Identification of ticks by 16S rDNA PCR	Detected tick-borne pathogens in ticks		
					SFG Rickettsia	A. phagocytophilum	Babesia spp.
1	83/F	A. testudinarium	Nymph	NA	-	A. phagocytophilum	-
2	46/M	A. testudinarium	Nymph	NA	R. tamurae	-	-
3	4/M	A. testudinarium	Nymph	A. testudinarium	-	-	-
4	NA	A. testudinarium	Nymph	A. testudinarium	-	-	-
5	65/M	A. testudinarium	Nymph	A. testudinarium	R. tamurae	-	-
6	74/M	A. testudinarium	Nymph	A. testudinarium	R. tamurae	-	-
7	58/F	A. testudinarium	Nymph	A. testudinarium	R. tamurae	-	-
8	52/F	A. testudinarium	Nymph	A. testudinarium	-	-	-
9	62/F	A. testudinarium	Nymph	A. testudinarium	-	-	-
10	60/M	A. testudinarium	Nymph	A. testudinarium	-	-	-
11	55/F	A. testudinarium	Nymph	A. testudinarium	-	-	-
12	30/F	A. testudinarium	Nymph	A. testudinarium		-	B. microti
13	71/M	A. testudinarium	Nymph	A. testudinarium	-	-	-
14	64/M	A. testudinarium	Adult (female)	NA	-	-	-
15	NA	A. testudinarium	Adult (female)	A. testudinarium	Ca. R. jingxinensis	-	-
16	60/F	A. testudinarium	Adult (female)	A. testudinarium	-	-	-
17	54/F	A. testudinarium	Adult (female)	A. testudinarium	R. tamurae	-	-
		A. testudinarium	Adult (male)	A. testudinarium	R. tamurae	-	-
18	53/F	A. testudinarium	Adult (male)	A. testudinarium	R. tamurae	-	-
19	78/F	A. testudinarium	Adult (male)	A. testudinarium	R. tamurae	-	B. gibsoni
20	60/F	H. longicornis	Nymph	H. longicornis	-	-	-
		H. longicornis	Nymph	NA	-	-	-
21	83/F	H. longicornis	Nymph	H. longicornis	Ca. R. jingxinensis	-	-
22	72/F	H. longicornis	Nymph	H. longicornis	-	-	-
23	76/F	H. longicornis	Adult (female)	H. longicornis	-	-	-
		H. longicornis	Adult (female)	H. longicornis	-	-	-
24	77/F	H. longicornis	Adult (female)	H. longicornis	Ca. R. jingxinensis	-	-
25	5/M	H. longicornis	Adult (female)	H. longicornis	-	-	B. microti
26	54/F	H. longicornis	Adult (female)	H. longicornis	-	-	-
27	77/F	I. nipponensis	Nymph	I. nipponensis	-	-	-
28	72/M	I. nipponensis	Adult (female)	I. nipponensis	-	-	-
29	53/F	I. nipponensis	Adult (female)	I. nipponensis	-	A. phagocytophilum	-
30	81/F	I. nipponensis	Adult (female)	I. nipponensis	R. monacensis	A. phagocytophilum	-

Molecular detection of tick-borne pathogens in ticks removed from humans

We examined 33 ticks for the detection of tick-borne pathogens using pathogen-specific nested PCR. The presence of tick-borne pathogens was detected in 16 ticks. From the results, 12 ticks (36.4%) tested positive for spotted fever Rickettsia, namely R. monacensis (1 of 33, 3.0%), R. tamurae (8 of 33, 24.2%), and Candidatus Rickettsia jingxinensis (3 of 33, 9.1%). Three ticks (9.1%) were positive for A. phagocytophilum, while another three ticks (9.1%) were positive for either B. gibsoni (1 of 33, 3.0%) or B. microti (2 of 33, 6.0%) (Table 3). All ticks were negative for Borrelia spp., Bartonella spp., and C. burnetii.

Table 3
Detection of tick-borne pathogens in ticks by pathogen-specific nested PCR

Detected pathogens	Positive tick numbers	/Total numbers	PCR positivity (%)
Spotted fever group Rickettsia species	12	/33	36.4
R. monacensis	1	/33	3.0
R. tamurae	8	/33	24.2
Candidatus Rickettsia jingxinensis	3	/33	9.1
Anaplama phagocytophilum	3	/33	9.1
Babesia species	3	/33	9.1
B. gibsoni	1	/33	3.0
B. microti	2	/33	6.0
Borrelia species	0	/33	0
Bartonella species	0	/33	0
Coxiella burnetii	0	/33	0

Of the three A. phagocytophilum-positive ticks, one tick was identified as A. testudinarium, and two ticks were identified as I. nipponensis. Of the 12 SFG Rickettsia-positive ticks, nine ticks were identified as A. testudinarium, two ticks were identified as H. longicornis, and one tick was identified as I. nipponensis. The presence of R. tamurae was identified only in A. testudinarium tick. Of the three ticks detected with Babesia spp., two ticks were A. testudinarium and one tick was H. longicornis. Among the 33 ticks, one I. nipponensis (adult female) was co-infected with A. phagocytophilum and R. monacensis. In addition, co-infections of R. tamurae and Babesia spp. were identified in A. testudinarium (adult male) that presented in Table 4.

Sequencing and phylogenetic analysis

The positive PCR products were sequenced and the sequencing results were aligned with the sequences obtained from the GenBank database to identify known sequences with a high degree of similarity using ClustalW. The neighbor-joining tree was constructed using the Kimura 2-parameter model (1,000 bootstrap replicates).

The partial ankA sequences obtained from A. phagocytophilum positive-tick demonstrated 99% similarity with A. phagocytophilum (accession no. KJ677106 and KT986059, 98% bootstrap support, Fig. 1A). The partial ankA sequences formed a cluster with the A. phagocytophilum strains isolated from humans in the ROK. The partial groEL sequences obtained from A. phagocytophilum-positive ticks demonstrated 99% similarity with the A. phagocytophilum strain isolated from humans and dogs in the ROK (accession no. KU519286, 66% bootstrap support, Fig. 1B).

The partial 17 kDa, ompA, and gltA sequences obtained from SFG Rickettsia-positive ticks showed 99–100% similarity with R. tamurae, R. monacensis, and Ca. R. jingxinensis. A phylogenetic analysis grouped the partial gltA sequences with R. tamurae (accession no. KT753273, 86% bootstrap support, Fig. 1C), R. monacensis (accession no. NZ LN794217, 92% bootstrap support, Fig. 1C), and Ca. R. jingxinensis (accession no. KT899089, 76% bootstrap support, Fig. 1C).

The partial 18S rDNA sequences obtained from two Babesia species-positive ticks (Tick 12 and Tick 25) showed 99% similarity with a B. microti strain isolated from humans in the USA and a tick in China (accession no. KU204794 and LC314655, 100% bootstrap support, Fig. 1D). Another partial 18S rDNA sequence obtained from Tick 19 had 99% similarity with B. gibsoni and 100% similarity with Babesia spp., which were clustered with a B. gibsoni strain isolated from a boar in China (accession no. JX962780, 100% bootstrap support, Fig. 1D) and Babesia spp. from a tick in Japan (accession no. LC169083, 96% bootstrap support, Fig. 1D).

Discussion

Recently, the risk of tick-borne disease has been associated with exposure to ticks from increasing outdoor activity. This study was performed to detect and identify the tick-borne pathogens in ticks removed from tick-bitten humans. We classified 33 ticks into three species: A. testudinarium (20, 60.6%; 7 adults and 13 nymphs) was the most common followed by H. longicornis (9, 27.3%; 5 adults and 4 nymphs) and I. nipponensis (4, 12.1%; 3 adults and 1 nymph). According to a tick survey study conducted by the KCDC (Korea Centers for Disease Control and Prevention) from 2013 to 2015, H. longicornis was the most dominant species (88.9%), followed by H. flava, I. nipponensis, I. persulcatus, H. japonica, A. testudinarium, and I. granulatus when ticks were collected from the vegetation and forests in the ROK using dry-ice bait traps and a flagging method (14). Interestingly, our results showed that when ticks were collected from tick-bitten humans, A. testudinarium was the most common.

For the molecular detection of tick-borne pathogens, we performed pathogen-specific N-PCR to detect the DNA of the tick-borne pathogens, namely SFG Rickettsia, A. phagocytophilum, Borrelia spp., Bartonella spp., Babesia spp., and C. burnetii. Three tick samples (3 of 33, 9.1%) were positive for A.

phagocytophilum DNA, 12 tick samples (12 of 33, 36.4%) were positive for R. monacensis, R. tamurae or Ca. R. jingxinensis DNA, and three ticks (3 of 33, 9.1%) were positive for B. gibsoni or B. microti DNA. Previous studies that investigated the prevalence of tick-borne infectious agents in ticks collected by dragging and flagging grass vegetation in the ROK showed that A. phagocytophilum was detected in 1.9% of H. longicornis ticks (15) and 0.1% of I. persulcatus ticks, and Rickettsia spp. were detected in 1.7% of H. longicornis ticks (16). One study reported that a pool of H. longicornis, H. flava, and I. nipponensis ticks collected by dragging vegetation in the ROK were positive for the Rickettsia spp. 17 kDa antigen (60/311, 19.3%) and ompA gene (53/311, 17.04%) (17). In the present study, the infection prevalence of Rickettsia species (R. monacensis, R. tamurae, and Ca. R. jingxinensis) and A. phagocytophilum in the ticks collected from humans was higher than that of ticks collected from the vegetation. Thus, we suggest that further study is needed to compare the infection prevalence of tick-borne pathogens, including Rickettsia spp., A. phagocytophilum, and Babesia between ticks isolated from humans and ticks collected from grass vegetation.

A. phagocytophilum infection was first reported with serological evidence from humans in 2002, and it is currently the most frequently reported tick-borne bacterial infection in the ROK (18). The detection of Anaplasma spp. in ticks from grazing cattle collected from all ROK provinces has been reported (19). Another study confirmed a human granulocytic anaplasmosis (HGA) with A. phagocytophilum in a patient from the ROK who had a history of tick bites, clinical symptoms, and positive laboratory findings (20). The present results showed that A. phagocytophilum was detected in A. testudinarium and I. nipponensis ticks. The amplicon sequences of the partial ankA gene in A. testudinarium (Tick 1) and I. nipponensis (Tick 29 and Tick 30) demonstrated more than 99% similarity. In the phylogenetic analysis, the sequences of the ankA gene from different types of ticks clustered together, showed > 99% similarity with A. phagocytophilum strains isolated from humans in the ROK (Fig. 1A).

The first isolation of R. monacensis from ticks in the ROK was reported in 2013 (21). A previous study from the ROK reported that I. nipponensis was infected with the human pathogen R. monacensis and that H. longicornis and H. flava were infected with unknown SFG Rickettsia pathogens (17). Our results confirmed the presence of R. monacensis in I. nipponensis ticks removed from humans. In addition, our results indicated that I. nipponensis ticks are most likely the vectors responsible for transmitting R. monacensis infections in the ROK. Therefore, further studies are needed to determine the role of I. nipponensis in the transmission of the R. monacensis pathogen to humans; the blood of patients bitten by I. nipponensis ticks and the ticks themselves should be investigated for the presence of R. monacensis.

R. tamurae was first isolated from A. testudinarium ticks collected in Japan in 1993. R. tamurae was formally identified as a novel species by genetic and phylogenetic analyses in 2006 (22). In 2011, the first case of human infection was confirmed using molecular and serological analyses in Japan (23). The presence of SFG Rickettsia including R. tamurae was found in Amblyomma and Dermacentor ticks in Thailand (24) and in Haemaphysalis ticks in Peninsular Malaysia (25). In addition, R. tamurae was found in Amblyomma ticks from an area endemic for Brazilian spotted fever in Brazil (26). Supporting these previous studies, our results showed the presence of R. tamurae in A. testudinarium ticks.

The presence of a potentially novel species of Ca. R. jingxinensis was proposed in H. longicornis nymphs from Jingxin in Northeastern China in 2016 (27) and was detected in H. longicornis ticks in Xi'an, China in 2017 (28). In the ROK, the pathogenicity of Ca. R. jingxinensis is not clear. Therefore, a further assessment of the potential pathogenicity in humans and animals is needed.

There have been no previous reports of R. tamurae or Ca. R. jingxinensis from ticks in the ROK; here, we report the first identification of R. tamurae and Ca. R. jingxinensis in ticks obtained from tick-bitten humans.

Babesia was first discovered in animals by Babes in 1988, and more than 100 species have been identified. In the ROK, Babesia spp. have been isolated from cattle and other mammals (raccoon, deer, and badger) since the 2000s (29–31). Babesia spp. are mainly carried by Ixodes ticks. Previous studies using ticks collected from grass and vegetation in the ROK reported that H. longicornis was the most common tick species infected with Babesia (16, 19). Our results showed that B. microti was found in both H. longicornis and A. testudinarium. In the USA, the primary vector for the transmission of B. microti to humans is the tick Ixodes scapularis in the nymphal stage (32). The present results suggest that further study is needed to determine the type of ticks that are the vectors for the transmission of B. microti to humans in the ROK.

B. gibsoni was first identified in nymphs of Rhipicephalus sanguineus ticks from infected dogs in Asia (33). B. gibsoni was detected in A. testudinarium ticks in this study. The first case of human babesiosis (KO1) was reported in 2007 in the ROK, and it was highly related to Chinese ovine Babesia spp. (12). Based on the phylogenetic analysis of the 18S rDNA gene in our study, the pathogen clustered with a group of Babesia spp., isolated from a tick in Japan, which was diverged from the KO1 strain (Fig. 1D). The present results indicate that Babesia spp. may vary based on their geographical distributions.

Further investigation is needed to determine the difference between pathogens found in ticks isolated from humans and ticks collected from grass vegetation. In addition, transmission studies should be conducted to determine whether the pathogens found in ticks are the same as those found in humans bitten by those ticks. To confirm the transmission of pathogens from ticks to humans, serological testing on the blood of tick-bitten patients and their ticks will be necessary. Further experiments and correlation analysis using the blood samples of tick-bitten humans and ticks isolated from them may help predict the transmission of tick-borne diseases.

Conclusions

In conclusion, we confirmed three tick species carrying tick-borne pathogens; the most common species was A. testudinarium followed by H. longicornis and I. nipponensis. These ticks were positive for SFG Rickettsia, A. phagocytophilum, and Babesia. This was the first report of the presence of R. tamurae and Ca. R. jingxinensis in ticks removed from tick-bitten humans in the ROK.

Abbreviations

ROK
Republic of Korea
PCR
polymerase chain reaction
SFG
spotted fever group
IRB
institutional review board
PBS
phosphate-buffered saline
PC/SM
penicillin and streptomycin
ompA
outer membrane protein A gene
gltA
citrate synthase gene
17 kDa
17 kDa protein gene
groEL
heat shock protein gene
ankA
ankyrin-related protein gene
pyrG
CTP synthase gene
ITS
16S-23S internal transcribed spacer region
IS1111
htpAB-associated repetitive element
C-PCR
conventional PCR
N-PCR
nested PCR

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics in Human Research Committee of Chosun University Hospital under an institutional review board (IRB), which approved all the experiments that used ticks removed from tick-bitten humans (approval no. CHOSUN NON2019-001). The IRB has been approved without written consent for the use of ticks and not human subject.

Consent for publication

Not applicable

Availability of data and materials

The data supporting the conclusions of this article are included within the article.

Competing interests

The authors declare that they have no competing interests.

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Contributions

MS Bang performed the investigation and drafted the manuscript. CM Kim contributed the methodology and revised the manuscript. SH Pyun contributed the investigation. DM Kim contributed the conceptualization, supervision and revised the manuscript. NR Yun supervised. All authors read and approved the final manuscript.

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Figures

Figure 1

Phylogenetic trees based on partial nucleotide sequences obtained from A. phagocytophilum-, spotted fever group Rickettsia-, and Babesia-positive ticks in this study and from GenBank. (A) 560 bp of the ankA gene (B) 330 bp of the groEL gene sequences for A. phagocytophilum (C) 420 bp of the gltA gene sequences for SFG Rickettsia (D) 370 bp of the 18S rRNA gene sequences for Babesia species

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