First Report for the Seasonal and Annual Prevalence of Flea-Borne *Bartonella* from Rodents and Soricomorphs in the Republic of Korea

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Abstract

Rodents and soricomorphs are animal hosts of fleas and associated zoonotic microbial pathogens. A total of 4,889 small mammals were collected from Gyeonggi and Gangwon Provinces, Republic of Korea, from 2008 through 2010, including: *Apodemus agrarius* (4,122, 84.3%), followed by *Crocidura lasiura* (282, 5.8%), *Microtus fortis* (257, 5.3%), *Myodes regulus* (77, 1.6%), *Micromys minutus* (71, 1.5%), *Mus musculus* (63, 1.3%), and 4 other species (17, 0.3%). A total of 1,099 fleas belonging to 10 species and 7 genera were collected. *Ctenophthalmus congeneroides* (724, 65.9%) was the most commonly collected flea, followed by *Stenoponia sidimi* (301, 27.4%), *Neopsylla bidentatiformis* (29, 2.6%), and *Rhadinopsylla insolita* (25, 2.3%). The remaining species accounted for only 1.8% (20, range 1–6) of all fleas collected. The 2 dominant flea species, *C. congeneroides* and *S. sidimi*, showed an inverse seasonal pattern, with higher populations of *C. congeneroides* from January–September, whereas *S. sidimi* was more frequently collected during October–December. The overall flea infestation rates (FIR) and flea indices (FI) were 14.1% and 0.22, respectively, and were highest during April–June (19.7% and 0.30, respectively). A total of 735 of the 1,099 fleas were assayed for the detection of *Bartonella* spp. by PCR using *Bartonella*-specific primers, of which 515 were positive for *Bartonella*, with an overall maximum likelihood estimate (MLE) of 700.7/1,000. The highest MLE values were observed during April–June (899.2) and July–September (936.2) trapping periods and, although lower, were similar for January–March (566.7) and October–December (574.1). *C. congeneroides* demonstrated high MLEs for all seasons (range 752.5–934.8), while *S. sidimi* was positive for *Bartonella* only during January–March (MLE = 342.1) and October–December (MLE = 497.2) collection periods. Continued long-term surveillance of small mammals and associated ectoparasites is needed to improve our understanding of the prevalence of *Bartonella* spp. in fleas and the role of fleas in the zoonotic maintenance and transmission of *Bartonella* to humans.

Key Words: Flea—*Bartonella*—Prevalence—*Apodemus agrarius*—*Ctenophthalmus congeneroides*—*Stenoponia sidimi*.

Introduction

OVER THE PAST FEW DECADES, there has been a reemergence of zoonotic vector-borne pathogens that pose medical and veterinary health risks. Small mammals, *i.e.*, rodents and soricomorphs, are hosts for known and yet-to-be described zoonotic pathogens, whereas their associated ec-

toparasites, *i.e.*, fleas, mites, and ticks, serve to maintain and transmit pathogens in natural host populations and incidentally to domestic animals and man (Gage 2005).

In the Republic of Korea (ROK), rodents and soricomorphs are animal hosts to a number of zoonotic pathogens (*i.e.*, *Rickettsia*, *Ehrlichia*, *Anaplasma*, *Bartonella*, and *Borrelia* spp.), whereas fleas, ticks, and mites serve as vectors (Park et al.

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1992, Kee et al. 1994, Chae et al. 2003, Lee et al. 2003, Kim et al. 2005). A survey of fleas collected from rats was first reported in Korea by Kobayashi (1931). In subsequent surveys, many flea species were observed and associated with natural hosts (Nagahana 1937, 1938, 1954, Tipton et al. 1972, Hong 1994). Recently, a preliminary investigation of zoonotic bacterial pathogens showed that the prevalence of *Bartonella* spp. from ticks, mites, and tissues of small mammals was relatively high, compared to other bacterial pathogens (Kim et al. 2005). In addition, *Rickettsia typhi* and *R. felis* were detected by PCR in fleas collected from rodents and soricomorphs (Kim et al. 2010, Ko et al. 2011).

Members of the genus *Bartonella* are Gram-negative obligate bacteria of mammalian red blood cells. Some species of *Bartonella* are the etiological agents of human and animal diseases that vary from mild-to-severe febrile illnesses, including endocarditis, neuroretinitis, and bacillary angiomatosis (Tappero et al. 1993, Kerkhoff et al. 1999, Tsukahara et al. 2000, Fenollar et al. 2005, Morway et al. 2008). There have been many studies related to the identification of *Bartonella* spp. in rodents worldwide. On the basis of these and other studies, it was shown that flea control resulted in the reduction in the prevalence of *Bartonella* in rodents, supporting the importance of their role in the maintenance and transmission of *Bartonella* bacteria among host populations and the potential for incidental transmission to domestic animals and humans (Jardine et al. 2006, Morway et al. 2008). In support of this, *Bartonella* species that were associated with rodent hosts also were observed to be associated with illnesses in humans (Welch et al. 1999, Comer et al. 2001, Smith et al. 2002, Kosoy et al. 2003, Iralu et al. 2006, Morway et al. 2008). Therefore, vector- and rodent-borne disease surveillance is critical to identify associated health threats and risks to reduce the potential for transmission of these zoonotic pathogens to humans.

Ultimately, comprehensive infectious disease surveillance programs that identify the diversity of hosts and their associated ectoparasites and the potential for human exposure to zoonotic vector-borne disease agents will provide a better understanding of disease maintenance cycles in nature and for predicting the emergence of infectious diseases and transmission to man (Nieto et al. 2007). Annual environmental factors, especially reforestation and rainfall patterns, and warming annual climatic trends over the past few decades, affect host and associated ectoparasite populations (Chilton et al. 2000, Chae et al. 2008). Therefore, multiple-year surveillance of hosts, ectoparasites, and associated pathogens is essential to the understanding of zoonotic pathogen maintenance cycles and predicting the emergence of infectious diseases of veterinary and medical importance.

In this study, fleas were collected from small mammals and assayed by PCR for the detection of *Bartonella* to determine host associations, seasonal and annual prevalence of fleas, and the maximum likelihood estimations (MLE) of *Bartonella* in fleas collected from small mammals from 2008 through 2010.

Materials and Methods

Samples and sampling sites

Fleas were collected from small mammals (rodents and soricomorphs) captured at US military installations and US- and ROK-operated training sites in Gyeonggi (Paju, Pocheon, Osan, Suwon, and Pyeongtaek) and Gangwon (Cheolwon) Provinces from March, 2008, through December, 2010 (Fig. 1). Small mammal trapping was conducted at rice paddies, military cantonment and fighting position sites, training area perimeters, intermittent streams, ponds, and areas that were infrequently traveled by vehicles or used for troop patrols and vehicular maneuver activities (Kim et al. 2011). Small mammal trapping was conducted as part of the US military hantavirus surveillance program, which consisted of surveying rodent-infested habitats within military sites. The primary vegetation consisted of tall grasses with various proportions of herbaceous vegetation and shrubs, and small groves of young deciduous trees with grasses and shrubs as understory (Kim et al. 2011). Surveys were conducted quarterly from January–March, April–June, July–September, and October–December. Collapsible live-capture Sherman® traps (7.7× 9×23 cm; H.B. Sherman, USA) baited with crackers and peanut butter, were set out during the daytime and checked the following morning. Nonabsorbent cotton balls were placed in the traps during spring and winter months to maintain small mammal temperatures and therefore reduce their mortality due to low nighttime temperatures. Traps positive for small mammals were numbered sequentially, placed in secure shipping containers, and transported to Korea University, Seoul. Small mammals were given a unique number, anesthetized, identified to species by morphological methods, sexed, and euthanized by cardiac puncture in accordance with Korea University animal use protocol (O'Guinn et al. 2010). For each small mammal, fleas were removed and placed individually in cryovials containing 100% ethyl alcohol. Each cryovial was labeled sequentially by host, and then transported to the 5th Medical Detachment, 168th Multifunctional Battalion, 65th Medical Brigade, Yongsan Army Garrison, Seoul, where they were identified to species and sex under a stereomicroscope using conventional taxonomic keys (Hopkins and Rothschild 1953, 1956, Hong 1994).

DNA extraction and PCR

Total genomic DNA was extracted from 735 individual fleas (n=250, 235, and 250 for 2008, 2009, and 2010, respectively) for the detection of *Bartonella*-specific DNA. Each flea was homogenized using a sterile Beadbeater TissueLyser II (Qiagen, Germany) with 180 μL of lysis buffer, 20 μL of proteinase K, and 5-mm stainless steel beads at 30 frequencies/s for 5 min, incubated overnight at 56°C, and then centrifuged at 20,000×g for 15 min at room temperature. After centrifugation, the supernatant was used for DNA extraction using DNeasy Tissue Kits (Qiagen, Germany). Two sequential PCRs for the detection of the RNA polymerase β-subunit (*rpoB*) gene for *Bartonella* were conducted using 2 sets of primers (Table 1). The PCRs were carried out in a 25-μL reaction volume containing 10–100 ng for the 1st PCR and 1 μL of the 1st PCR product for the 2nd PCR of DNA template, 10×PCR buffer (Takara, Korea), 2.5 mM of each deoxyribonucleotide triphosphate (dNTP), 10 pmol of primers, and 1 unit DNA *Taq* polymerase (Takara, Korea). PCR was performed in a PTC-200 thermal cycler with the following conditions: 1st PCR, initial denaturation for 5 min at 94°C, followed by 30 cycles (94°C for 45 s, 57°C for 45 s, and 72°C for 45 s) with a final extension for 5 min at 72°C; 2nd PCR, initial denaturation for 5 min at 94°C, followed by 25 cycles (94°C for 30 s, 60°C for
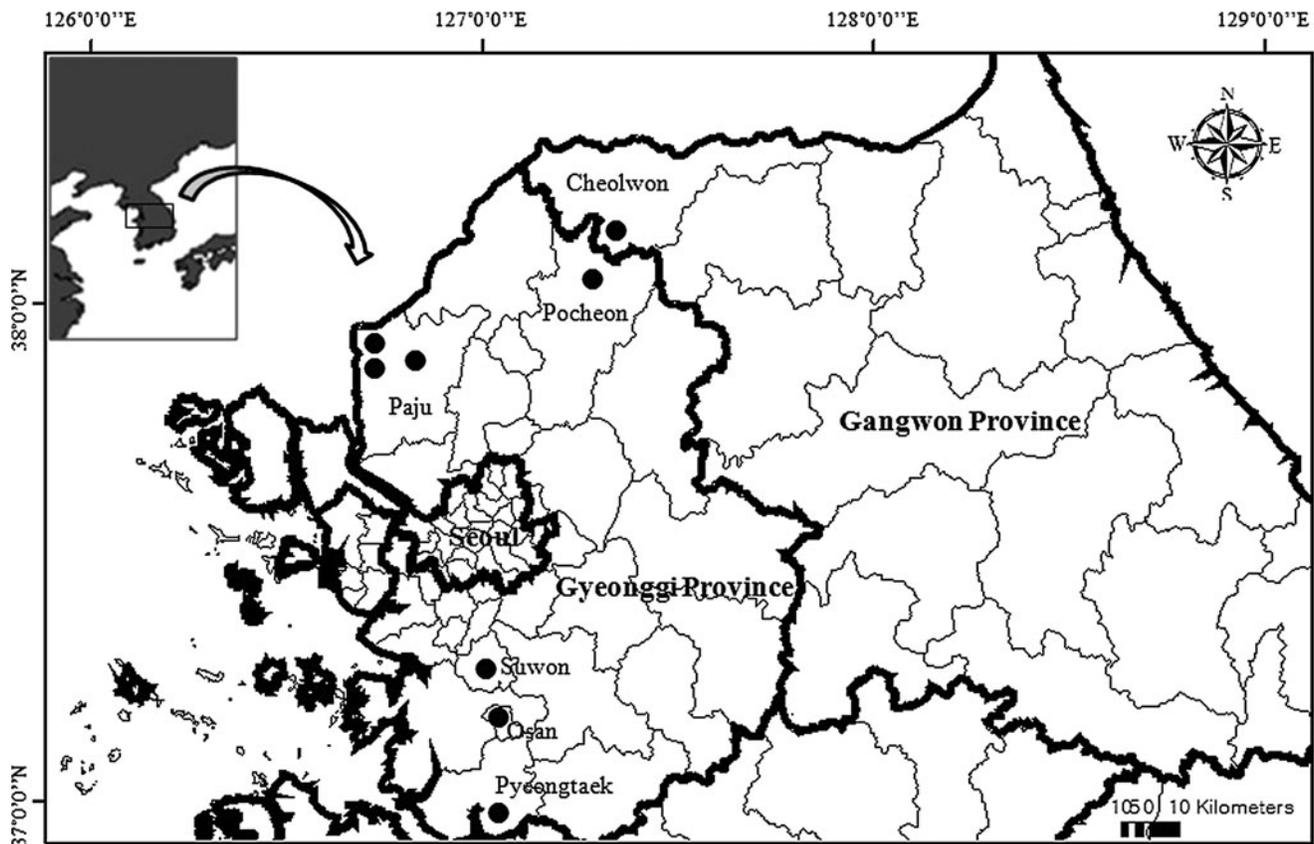
FIG. 1. Small mammal collection sites (dots) in Gyeonggi and Gangwon Provinces, Republic of Korea from 2008 through 2010. General site locations were: Warrior Base, Twin Bridge Training Areas and Dragon Head (Paju City), Nightmare Range (Pocheon County), Osan Air Base (Osan City), Suwon Air Base (Suwon City), and Camp Humphreys (Pyeongtaek City) in Gyeonggi Province; Chipori Range (Cheolwon County) in Gangwon Province.

30 s, and 72°C for 30 s) with a final extension for 5 min at 72°C. The PCR products were resolved by electrophoresis on a 1.5% agarose gel and detected using ethidium bromide.

Cloning, sequencing, and phylogenetic analysis

PCR products were purified with QIAquick Gel Extraction kits (Qiagen, Germany), and then cloned with pGEM®-T Easy Vectors (Promega Corp., USA) according to the manufacturer's protocols. Plasmid DNA for sequencing was again purified using the Wizard® *Plus* SV Minipreps DNA Purification System (Promega Corp., USA). Purified recombinant plasmid DNA was sequenced using T7 and SP6 promoter primers with an automatic sequencer (ABI 3730xl capillary DNA sequencer, USA). All sequences were manually edited at least 5 times on AlignIR program version 2.1 (LI-COR Inc., USA). Analyses of

the sequences were comparatively completed using a BLAST search for *Bartonella rpo*B sequences in GenBank. A multiple sequence alignment was conducted using BioEdit version 7.0.9.0 (Fig. 2; Hall 1999). The phylogenetic relationships of the *rpo*B sequences were constructed using the neighbor-joining method (Saitou and Nei 1987) under the Kimura 2-parameter mode (Kimura 1980) (Fig. 3). As reference sequences, corresponding *rpo*B sequences were chosen from GenBank database (Figs. 2 and 3). Also, corresponding *rpo*B sequences of *Rickettsia typhi*, *Ehrlichia canis*, and *Anaplasma phagocytophilum* were selected to root the phylogenetic tree as outgroups. Confidence in estimated relationship was determined using the bootstrap approach (Felsenstein 1985) obtained through 1,000 replicates incorporating the same model as above. Bootstrap analysis and phylogeny reconstruction were conducted using MEGA version 4.0 (Tamura et al. 2007).

TABLE 1. NESTED PCR PRIMERS USED FOR THE DETECTION OF THE RNA POLYMERASE BETA SUBUNIT (rpoB) GENE OF Bartonella spp. IN THIS STUDY

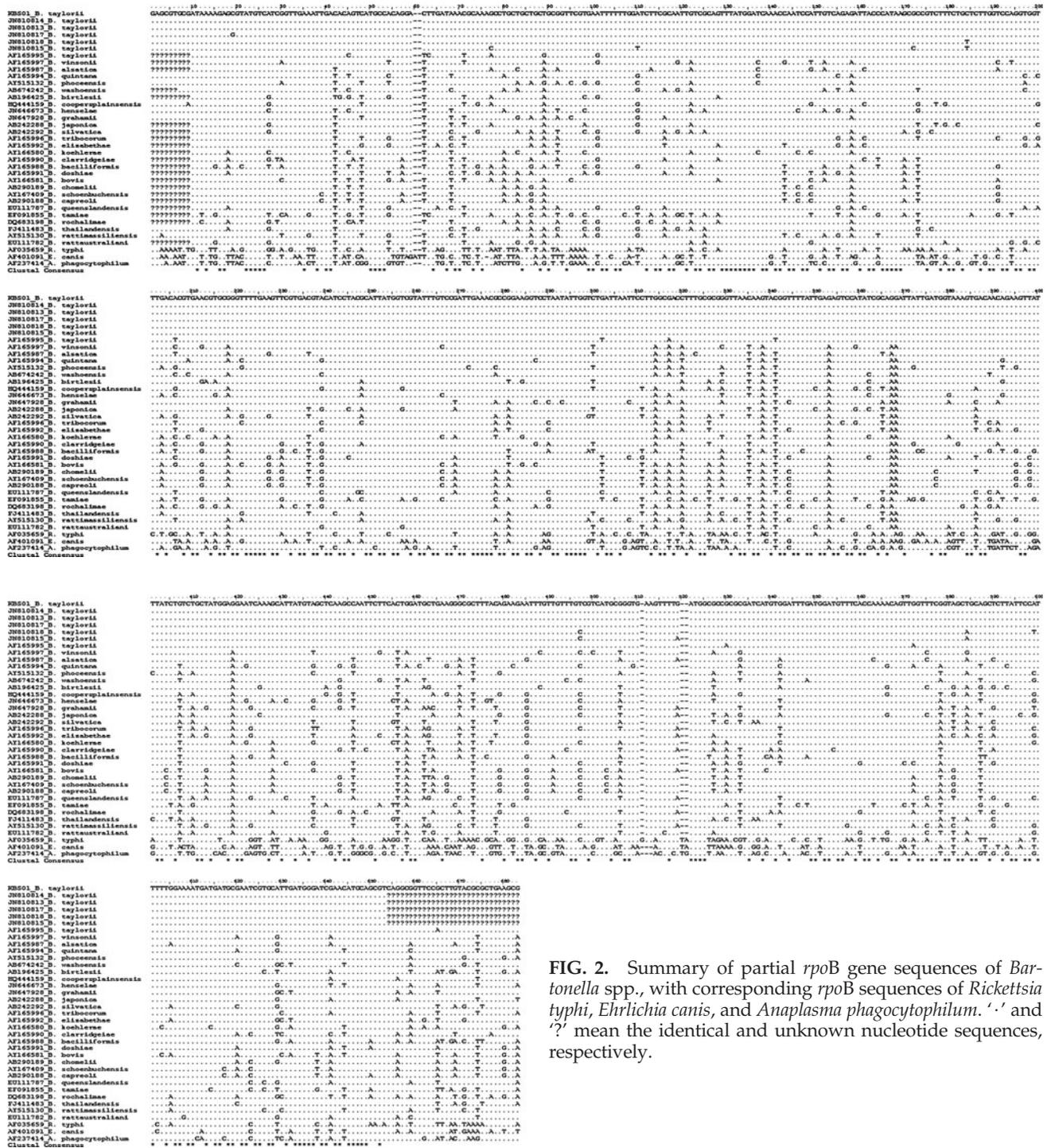| PCRs	Locus	Primers	Directions	Primer sequences (5′-3′)	Temp. (°C)[a]	References
1st	*rpo*B	1400F	Forward	CGC ATT GGC TTA CTT CGT ATG	57	Renesto et al. (2001)
		2300R	Reverse	GTA GAC TGA TTA GAA CGC TG		
2nd	*rpo*B	1400F	Forward	CGC ATT GGC TTA CTT CGT ATG	60	Renesto et al. (2001)
		rpoB IR	Reverse	TTC CCG TAC CAA CAA ATG G		This study

[a]Annealing temperature in PCR reactions.

FIG. 2. Summary of partial *rpo*B gene sequences of *Bartonella* spp., with corresponding *rpo*B sequences of *Rickettsia typhi*, *Ehrlichia canis*, and *Anaplasma phagocytophilum*. '·' and '?' mean the identical and unknown nucleotide sequences, respectively.

Data analyses of prevalence

Flea infestation rates (FIR) and flea indices (FI) were calculated (Kim et al. 2010, Ko et al. 2011) as follows: FIR = (Number of mammals, by species, with fleas/Total number of mammals, by species) × 100; FI = Number of fleas collected from mammals, by species/Total number of mammals, by species. The MLE infection rates were calculated using the PooledInfRate Software version 4.0 (Biggerstaff 2006). In the traditional analysis of prevalence data using the MFIR, an assumption is required that when a pool is positive, then only

1 individual in that pool is positive. However, MLE methods do not require this assumption. Although we used 1 flea for each pool, the MLE method was applied in this study. For more detailed information, refer to Biggerstaff (2006).

Results

A total of 1,099 fleas were collected from 4,889 small mammals belonging to 10 species and 4 genera (Table 2). *Apodemus agrarius* (4,122, 84.3%) was the most frequently captured small mammals, followed by *Crocidura lasiura* (282,
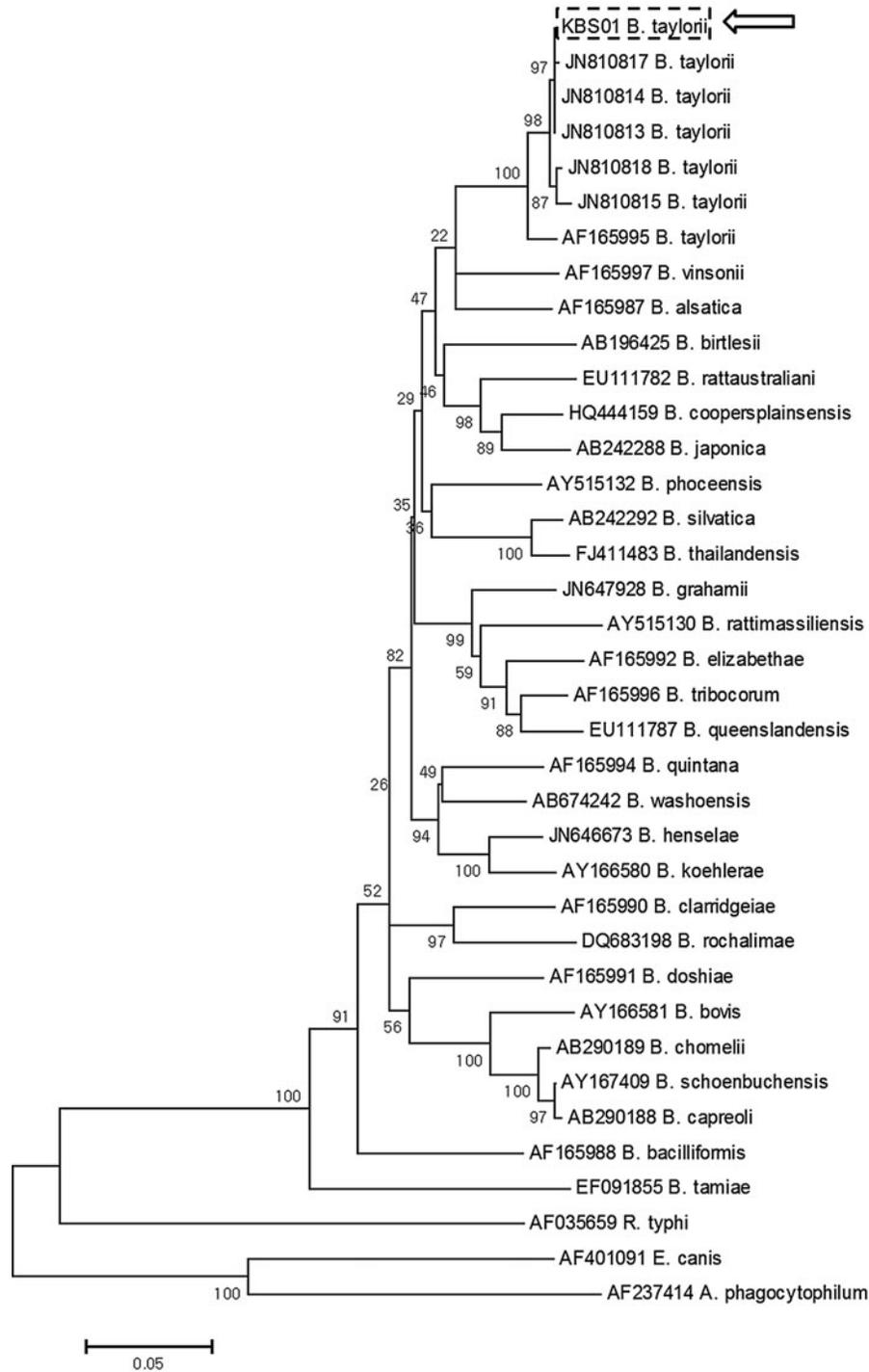
FIG. 3. The phylogenetic tree of partial *rpo*B gene sequences of *Bartonella taylorii*, and the other *Bartonella* spp. As outgroups, corresponding *rpo*B sequences of *Rickettsia typhi, Ehrlichia canis,* and *Anaplasma phagocytophilum* were used. The tree was rooted using 3 different sequences. The arrow indicates a genotype (KBS01 *B. taylorii* strain) detected in this study.

5.8%), *Microtus fortis* (257, 5.3%), *Myodes regulus* (77, 1.6%), *Microtus minutus* (71, 1.5%), and *Mus musculus* (63, 1.3%), whereas the remaining 4 species accounted for only 0.3% (17) of all small mammals collected. Overall, the FIR was 14.1%, ranging from 3.2% (*M. musculus*) to 66.7% (*Tamias sibiricus*) (Table 2). The overall FI for all mammals was 0.22 and highest from *T. sibiricus* (1.33), followed by *A. peninsulae* (0.50) and *Rattus norvegicus* (0.33) (Table 2). The overall mean number of

fleas collected from the small mammals was 1.6 (range 1.0–2.0) (Table 2).

Overall, the number of fleas was similarly collected during October–December (355, 32.3%), April–June (335, 30.5%), and January–March (312, 28.4%), whereas lower numbers were collected during July–September (97, 8.8%) (Table 3). For sites collected during all seasons and years (Paju, Pocheon, and Pyeongtaek), the FIRs and FIs were highest for Paju (21.1%,

TABLE 2. THE NUMBER AND PERCENT OF SMALL MAMMALS, NUMBER OF SMALL MAMMALS WITH FLEAS, NUMBER OF FLEAS, MEAN NUMBER OF FLEAS COLLECTED FROM EACH SPECIES, AND FLEA INFESTATION RATES (FIR) AND FLEA INDICES (FI) FOR SMALL MAMMALS COLLECTED AT US MILITARY INSTALLATIONS AND US- AND ROK-OPERATED TRAINING SITES IN GYEONGGI AND GANGWON PROVINCES, REPUBLIC OF KOREA, 2008–2010

Order	Family	Host species	No. (%) mammals captured	No. mammals with fleas	No. fleas collected	Mean no. fleas[a]	Flea infestation rates (%)	Flea index (FI)
Rodentia	Muridae	*Apodemus agrarius*	4,122 (84.3)	607	962	1.6	14.7	0.23
		Apodemus peninsulae	4 (<0.1)	2	2	1	50.0	0.50
		Mus musculus	63 (1.3)	2	2	1	3.2	0.03
		Micromys minutes	71 (1.5)	5	5	1	7.0	0.07
		Rattus norvegicus	3 (<0.1)	1	1	1	33.3	0.33
	Cricetidae	*Microtus fortis*	257 (5.3)	35	64	1.8	13.6	0.25
		Tscherskia triton	7 (0.1)	2	2	1	28.6	0.29
		Mydoes regulus	77 (1.6)	11	21	1.9	14.3	0.27
	Sciuridae	*Tamias sibiricus*	3 (<0.1)	2	4	2	66.7	1.33
Soricomorpha	Soricidae	*Crocidura lasiura*	282 (5.8)	20	36	1.8	7.1	0.13
	Total		4,889 (100.0)	687	1,099	1.6	14.1	0.22

[a]Mean number of fleas per infested mammal for each species (no. fleas collected/no. mammals with fleas).

0.35), whereas they were similar for Pocheon (12.9%, 0.19) and Pyeongtaek (12.5%, 0.21). Both FIRs and FIs for all fleas were similar and highest during April–June (19.7%, 0.30), October–December (17.4%, 0.28), and January–March (15.4%, 0.25) but much lower during July–September (4.4%, 0.08) (Fig. 4).

Ctenophthalmus congeneroides (724, 65.9%) was the most commonly collected flea from small mammals, followed by *Stenoponia sidimi* (301, 27.4%), *Neopsylla bidentatiformis* (29, 2.6%) and *Rhadinopsylla insolita* (25, 2.3%), whereas the remaining 6 species accounted for only 1.8% (20, range 1–6) of all fleas collected (Table 4). Except for *Doratopsylla coreana* and *Monopsyllus anisus*, all species were recorded from *A. agrarius* (Table 4). The 2 dominant flea species, *C. congeneroides* and *S. sidimi*, showed an inverse seasonal pattern, with higher populations observed for *C. congeneroides* during January–September, whereas *S. sidimi* was more frequently collected during October–December collection periods (Fig. 4).

Using *Bartonella* genus-specific PCR assays, *Bartonella* was detected in 515/735 (70.1%) of the fleas assayed. For the 2

most frequently collected species of fleas, *Bartonella* was detected in 84.7% (392/463) and 44.9% (115/256) of *C. congeneroides* and *S. sidimi*, respectively (Table 5). Overall, the highest MLE rates were observed during July–September (936.2), followed by April–June (899.2), October–December (574.1), and January–March (566.7). The MLEs of *C. congeneroides* were higher than that of *S. sidimi* for each of the survey periods. Overall, *A. agrarius* accounted for the greatest number of fleas collected (962/1099, 87.5%) (Table 4) and largest number of *Bartonella*-positive fleas (476/515, 92.4%).

Overall, annual FIRs for 2008–2010 were similar (range 12.6–20.6%), with FIs ranging from 0.19 to 0.35 (Table 6). The percentage of variation in the *Bartonella*-positive fleas (58.0–80.4%) was likely affected by differences in collection sites for each of the years and numbers of small mammals collected at each of the sites. In addition, it is difficult to extrapolate our findings to the rest of the ROK, due to geographic limitations of the present samplings. Latitudinal and longitudinal survey site surveillance in the ROK is needed to provide more

TABLE 3. NUMBER (%) OF SMALL MAMMALS CAPTURED, NUMBER OF SMALL MAMMALS WITH FLEAS, NUMBER AND MEAN NUMBER OF FLEAS COLLECTED, FLEA INFESTATION RATES (FIR), FLEA INDICES (FI), AND NUMBER (%) OF FLEAS COLLECTED SEASONALLY FOR EACH OF THE US MILITARY INSTALLATIONS AND US- AND ROK-OPERATED TRAINING SITES IN GYEONGGI AND GANGWON PROVINCES, REPUBLIC OF KOREA, 2008–2010

Provinces	Sampling sites	No. sites	No. (%) Mammals Captured	No. mammals with fleas	No. flea collected	Mean no. fleas[a]	Flea infestation rate (%)	Flea index (FI)	Jan–Mar (n=1,264)[b]	Apr–Jun (n=1,112)[b]	Jul–Sep (n=1,241)[b]	Oct–Dec (n=1,272)[b]
									No. (%) fleas collected			
Gyeonggi	Paju	3	722 (14.8)	152	255	1.7	21.1	0.35	46 (18.0)	15 (5.9)	2 (0.8)	192 (75.3)
	Pocheon	1	1,725 (35.3)	223	325	1.5	12.9	0.19	137 (42.2)	65 (20.0)	46 (14.2)	77 (23.7)
	Osan	1	26 (0.5)	9	12	1.3	34.6	0.46	0	12 (100.0)	0	0
	Suwon	1	12 (0.3)	5	7	1.4	41.7	0.58	0	7 (100.0)	0	0
	Pyeongtaek	1	2,316 (47.4)	290	485	1.7	12.5	0.21	114 (23.5)	236 (48.7)	49 (10.1)	86 (17.7)
Gangwon	Cheolwon	1	88 (1.8)	8	15	1.9	9.1	0.17	15 (100.0)	0	0	0
	Total	8	4,889 (100.0)	687	1,099	1.6	14.1	0.22	312 (28.4)	335 (30.5)	97 (8.8)	355 (32.3)

[a]Mean number of fleas per infested mammal for each species (no. fleas collected/no. mammals with fleas).
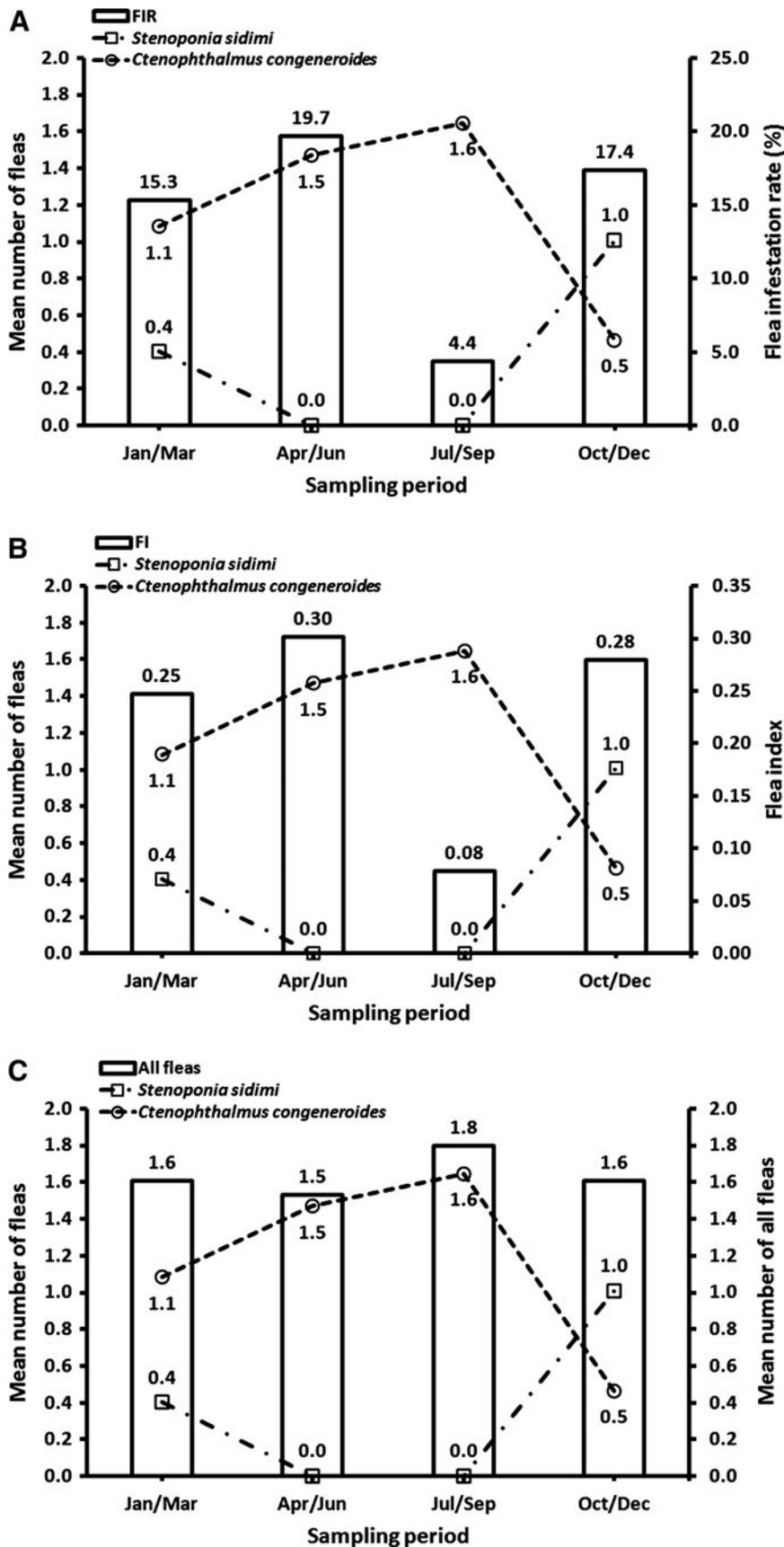[b]The number of small mammals captured.

FIG. 4. Flea infestation rates (FIR) (**A**), flea indices (FI) (**B**), mean number of all fleas (**C**), and mean number of fleas for *Ctenophthalmus congeneroides* and *Stenoponia sidimi*, collected from small mammals at US military installations and US- and ROK-operated training sites in Gyeonggi and Gangwon Provinces, Republic of Korea, 2008–2010.

TABLE 4. THE NUMBER (%) OF EACH FLEA SPECIES COLLECTED FROM INFESTED SMALL MAMMALS CAPTURED AT US MILITARY INSTALLATIONS AND US- AND ROK-OPERATED TRAINING SITES IN GYEONGGI AND GANGWON PROVINCES, REPUBLIC OF KOREA, 2008–2010

Host species	Stenoponia sidimi	Rhadinopsylla insolita	Rhadinopsylla concava	Ctenophthalmus congeneroides	Neopsylla bidentatiformis	Neopsylla specialis	Doratopsylla coreana	Hystrichopsylla microti	Monopsyllus indages	Monopsyllus anisus	Total (%)
Apodemus agrarius	285 (29.6)	20 (2.1)	2 (0.2)	622 (64.7)	25 (2.6)	4 (0.4)	0	3 (0.3)	1 (0.1)	0	962 (87.5)
Apodemus peninsulae	2 (100.0)	0	0	0	0	0	0	0	0	0	2 (0.2)
Mus musculus	1 (50.0)	0	0	1 (50.0)	0	0	0	0	0	0	2 (0.2)
Micromys minutes	0	0	0	4 (80.0)	0	1 (20.0)	0	0	0	0	5 (0.5)
Rattus norvegicus	0	0	0	1 (100.0)	0	0	0	0	0	0	1 (0.1)
Microtus fortis	1 (1.6)	1 (1.6)	0	62 (96.9)	0	0	0	0	0	0	64 (5.8)
Tscherskia triton	1 (20.0)	0	0	1 (20.0)	0	0	0	0	0	0	2 (0.2)
Myodes regulus	5 (23.8)	0	0	15 (71.4)	1 (4.8)	0	0	0	0	0	21 (1.9)
Tamias sibiricus	0	0	0	0	0	0	0	0	3 (75.0)	1 (25.0)	4 (0.4)
Crocidura lasiura	6 (16.7)	4 (11.1)	0	18 (50.0)	3 (8.3)	1 (2.8)	2 (5.6)	2 (5.6)	0	0	36 (3.3)
Total (%)	301 (27.4)	25 (2.3)	2 (0.2)	724 (65.9)	29 (2.6)	6 (0.5)	2 (0.2)	5 (0.5)	4 (0.4)	1 (0.1)	1,099

TABLE 5. THE NUMBER OF FLEAS POSITIVE FOR BARTONELLA AND MAXIMUM LIKELIHOOD ESTIMATION (MLE) OF BARTONELLA DETECTED IN FLEAS, BY SPECIES, USING GENUS-SPECIFIC PCR TECHNIQUES FOR FLEAS, COLLECTED FROM SMALL MAMMALS CAPTURED AT US MILITARY INSTALLATIONS AND US- AND ROK-OPERATED TRAINING SITES IN GYEONGGI AND GANGWON PROVINCES, REPUBLIC OF KOREA, 2008–2010

Flea species	No. fleas[a]	No. positive fleas[a]	MLE	No. positive fleas (MLE)			
				Jan–Mar (n=180)[b]	Apr–Jun (n=238)[b]	Jul–Sep (n=47)[b]	Oct–Dec (n=264)[b]
Ctenophthalmus congeneroides	463	392	846.7	76 (752.5)	212 (902.1)	43 (934.8)	61 (753.1)
Stenoponia sidimi	256	115	449.2	26 (342.1)	0	0[c]	89 (497.2)
Rhadinopsylla insolita	6	3	500	0[c]	0[c]	0[c]	3 (500.0)
Doratopsylla coreana	1	0	0	0[c]	0[c]	0[c]	0
Rhadinopsylla concava	1	1	—[c]	0[c]	0[c]	0[c]	1[c]
Hystrichopsylla microti	4	2	500	0	2[c]	0[c]	0
Monopsyllus indages	2	0	0	0	0[c]	0[c]	0[c]
Neopsylla bidentatiformis	2	2	—[c]	0[c]	0[c]	1[c]	1[c]
Total	735	515	700.7	102 (566.7)	214 (899.2)	44 (936.2)	155 (574.1)

[a] One flea per pool (totally 735 fleas or pools).
[b] The number of fleas tested for detection of Bartonella spp.
[c] Not applicable (N/A) in the MLE method.

TABLE 6. FLEA INFESTATION RATES (FIR), FLEA INDICES (FI), MEAN NUMBER OF FLEAS COLLECTED FROM SMALL MAMMALS, NUMBER OF BARTONELLA-POSITIVE FLEAS, AND THE MAXIMUM LIKELIHOOD ESTIMATIONS (MLE) FOR FLEAS COLLECTED FROM SMALL MAMMALS CAPTURED AT US MILITARY INSTALLATIONS AND US- AND ROK-OPERATED TRAINING SITES IN GYEONGGI AND GANGWON PROVINCES, REPUBLIC OF KOREA, 2008–2010

Status	2008	2009	2010	Overall
Flea infestation rates (FIR) (%)	13.0	12.6	20.6	14.1
Flea indices (FI)	0.19	0.21	0.35	0.22
Mean number of fleas	1.4	1.6	1.7	1.6
Number of *Bartonella*-positive pools (%)	201 (80.4)	169 (71.9)	145 (58.0)	172 (70.1)
Maximum likelihood estimations (MLE)	804.0	719.1	580.0	700.7

accurate information of seasonal and annual differences in FIRs, FIs, and MLEs as a result of climatic differences (*i.e.*, rainfall) for *Bartonella*-positive fleas.

One genotype was detected out of 3 different samples (2 *S. sidimi* and 1 *C. congeneroides* collected from *A. agrarius* in Gyeonggi Province; specimen voucher no. F09-007, 008, and 020) in the study. The sequence was deposited in GenBank (accession no. JX984664; strain KBS01). The 678-bp *rpo*B gene nucleotide sequences of the 3 samples were identical to *B. taylorii* (JN810813-4 with 95% query coverage). A consensus phylogenetic tree was reconstructed with reference and outgroup sequences (Fig. 3). To date, 5 species, *B. taylorii* (S. Ko, unpublished data), *B. grahamii* (S. Ko, unpublished data), *B. henselae* (Kim et al. 2009), *B. clarridgeiae* (Kim et al. 2009), and *B. elizabethae* (Kim et al. 2005), have been detected in the ROK.

Discussion

At the end of the Korean War (1950–1953), the Korean landscape was largely devoid of trees due to military activities and poverty, which restricted the development of small (*i.e.*, rodents, soricomorphs, and associated predators), medium (*i.e.*, raccoon dogs and leopard cats), and large (*i.e.*, water deer and wild boar) mammal populations that use forests for refuge. During the 1960s, a national tree planting policy was instituted by the ROK government, with nonagricultural lands (hills and mountains) now encompassing large expanses of groves of planted trees, *i.e.*, chestnuts, oaks, pines, larch, and volunteer trees (Lee and Lee, 2002). As Korea consists of 70% mountainous terrain, this greatly increased the potential for harborage of wild mammals, *i.e.*, water deer, wild boar, raccoon dogs, leopard cats, weasels, rodents, soricomorphs, and feral cats and dogs, and the associated ectoparasites, *i.e.*, fleas, ticks, and mites, that they harbor. Recently, *R. typhi* (Typhus group) and *R. felis* (Spotted fever group *Rickettsia*) were detected in fleas collected from small mammals in the ROK (Kim et al. 2010, Ko et al. 2011). However, identification and distribution of flea-borne pathogens, including *Bartonella*, has not been well documented in the ROK.

During recent years, a number of *Bartonella* species, which are etiological agents of many human and animal diseases, have been isolated and characterized, including a few model organisms for studying the evolution and ecology of vector-borne diseases (Jacomo et al. 2002). For examples, sandfly-borne *B. bacilliformis* (oroya fever) in a Peruvian Andean village, South America (Boulouis et al. 2005), louse-borne *B. quintana* (trench fever) in many countries of the world (Fournier et al. 2002), flea-borne *B. henselae* (cat scratch disease and bacillary angiomatosis) (Karem 2000), and other Bartonellae in humans. However, few studies have been conducted on *Bartonella* species in the ROK, compared with the numerous studies through the world. Although some of reports on the molecular detection of *Bartonella* from fleas of pets (Han et al. 2006), ticks, mites, and small mammals (Kim et al. 2005, Kim et al. 2009), this is the first report on the prevalence of flea-borne *Bartonella* from small mammals in the ROK.

A. agrarius was the most frequently captured mammal among 10 different small mammal species, and had the most diverse spectrum of fleas (8 of 10 species of fleas, including *C. congeneroides* and *S. sidimi*), as previously reported by others (Kim et al. 2010, Ko et al. 2011). These data implicate *A. agrarius* and associated fleas as hosts/vectors for the maintenance and transmission of *Bartonella* in the ROK. In general, *A. agrarius* is found throughout the ROK in a wide range of rural habitats and in tall grass habitats associated with parks and other areas in urban environments (Kim et al. 2011) and is the primary host for Hantaan virus in the ROK (Song et al. 2009, Kim et al. 2011). The habitats in rural farmlands and local and national parks increase the likelihood of human–flea contact, which increases the potential for transmission of flea-borne pathogens to human populations.

The seasonal distribution of FIRs was similar to those observed from previous studies (Walton and Hong 1978, Kim et al. 2010, Ko et al. 2011), except for June (Kim et al. 2010) and August to September (Ko et al. 2011). FIs showed similar seasonal patterns to FIRs, but the mean number of fleas showed an inverse pattern to FIRs. The two most commonly collected flea species, *C. congeneroides* and *S. sidimi*, demonstrated distinct seasonal distributions and prevalence of *Bartonella*. Kim et al. (2010) suggested that this might be due to host and/or flea seasonal reproductive differences. However, there are no clear studies to answer this question. In addition, positive fleas found in this study were observed mainly from fleas of *A. agrarius*, whereas those of the other species accounted for only minor proportion as a result of relatively low catches for those species.

Occasional flooding, due to seasonal monsoon rains and typhoons, may increase the movement and dispersal of small mammals and associated ectoparasites and zoonotic pathogens that they harbor (Kim et al. 2011). During some years, the rainy season extends from August through September resulting in periodic and occasional flooding of low-lying areas, while during other years the rainy period is much shorter. Also, global climatic change has been reported in the ROK with unknown effects on vegetation, wildlife, domestic animals, and humans. Vectors (*i.e.*, mosquitoes, fleas, lice, ticks, and mites) that harbor infectious agents are sensitive to climatic changes (*i.e.*, increasing temperature, relative humidity, sunshine hour, and precipitation) and may increase/decrease the potential transmission of zoonotic pathogens to domestic animals and humans (Chae et al. 2008). In addition, the accidental or intentional contact with vectors are increasing and pose serious

public health risks, particularly as a result of public recreation activities, agricultural and urban expansion, outdoor construction, military training/operations, or possibly, in the event of a natural disaster (Richards et al. 1997, Eisen et al. 2007, Kim et al. 2010, Ko et al. 2011). The present multiple-year prevalence study provides important information on the epidemiology of flea-related *Bartonella* pathogens in the ROK. However, long-term monitoring of at least 5–10 years and more detailed analyses of relationships between environmental factors, prevalence, distribution of zoonotic flea-borne pathogens, and anthropogenic disturbance are essential for understanding disease maintenance cycles and predicting the emergence of vector-borne diseases affecting veterinary and medical health under changing climatic conditions.

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