

## DETECTION OF *RICKETTSIA TYPHI* AND SEASONAL PREVALENCE OF FLEAS COLLECTED FROM SMALL MAMMALS IN THE REPUBLIC OF KOREA

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**ABSTRACT:** Fleas were collected from live-captured small mammals to identify potential flea-borne pathogens, seasonal prevalence of flea species, and host preference as part of the US military rodent-borne diseases surveillance program conducted at one US military installation and 10 military training sites, northern Gyeonggi Province, Republic of Korea. During 2003–04, 948 fleas (563 females and 385 males) were recovered from 2,742 small mammals (seven rodent and one insectivore species). *Apodemus agrarius* (striped field mouse) accounted for 88.9% (2,439/2,742) of the small mammals, followed by *Crocidura lasiura* (4.2%), *Mus musculus* (2.9%), *Microtus fortis* (2.2%), *Myodes regulus* (0.6%), *Micromys minutus* (0.5%), *Tscherskia triton* (0.5%), and *Rattus norvegicus* (0.3%). Small mammal infestation rates (number with fleas/number captured) ranged from 7.7% (*M. minutus* and *T. triton*) to 31.3% (*M. regulus*). Flea indices were highest for *M. regulus* (0.69/captured rodent), followed by *C. lasiura* (0.54), *M. fortis* (0.41), *A. agrarius* (0.34), and *R. norvegicus* (0.33). Overall, *Ctenophthalmus congeneroides* (51.3%) was more frequently collected, followed by *Stenoponia sidimi* (42.6%), *Rhadinopsylla insolita* (5.5%), *Neopsylla bidentatiformis* (0.4%), *Rhadinopsylla concava* (0.1%), and *Doratopsylla coreana* (0.1%). *Ctenophthalmus congeneroides* was more frequently collected from small mammals during the spring and summer, while *S. sidimi* was more frequently collected during the winter season. *Rickettsia typhi*, the causative agent of murine typhus, was detected in 3.2% of specimens (7/220 pools from 654 fleas; minimum field infection rate [number of positive pools/total number of fleas] was 1.1%).

**Key words:** *Ctenophthalmus congeneroides*, flea, Korea, *Rickettsia typhi*, *Stenoponia sidimi*.

### INTRODUCTION

Fleas are vectors of zoonotic pathogens of public health importance, including plague, murine typhus, and other flea-borne rickettsial pathogens (Mohr, 1951; Parola et al., 2005). The accidental or intentional introduction of flea-borne diseases poses serious public health risks, especially during military training exercises, natural disasters, or military operations where reservoir hosts (e.g., rodents and insectivores) and associated ectoparasites occur in high densities or hosts are displaced leaving ectoparasites behind (Richards et al., 1997; Eisen et al., 2007). In the Republic of Korea (ROK), small

mammals are reservoir hosts to a number of zoonotic pathogens that are transmitted by ticks, mites, and fleas, including murine typhus, scrub typhus, and other rickettsial diseases (Chu and Hong, 1958; Ahn and Soh, 1973; Hong et al., 1975; Walton and Hong, 1976; Lee et al., 1983; Hong and Shin, 1990; Kim et al., 2003). A survey of rat fleas was first reported in Korea by Kobayashi (1931), and subsequent surveys identified 37 species with associated host-parasite relationships (Nagahana, 1937a, b, 1938a, b, c, 1954; Tipton et al., 1972; Hong, 1994). Rodent-borne disease surveillance programs that identify ectoparasite diversity and potential human exposure to zoonotic vector-borne disease

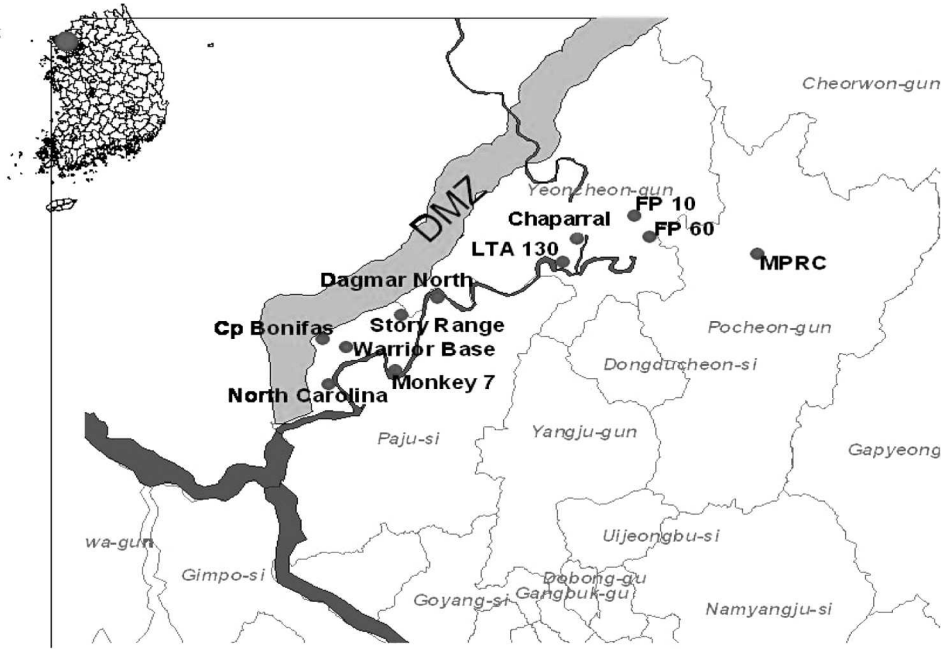


FIGURE 1. Geographic locations of small mammal collection sites: 10 US-operated and Republic of Korea's (ROK)-operated training sites and one USA military installation, northern Gyeonggi Province, ROK. North Carolina Range, Jangdan-myeon, Paju-si, Gyeonggi Province; Camp Bonifas, and Warrior Base, Gunnae-myeon, Paju-si, Gyeonggi Province; Monkey 7, and Story Range, Jindong-myeon, Paju-si, Gyeonggi Province; Dagmar North, Jeokseong-myeon, Paju-si, Gyeonggi Province; FP 10 (Firing Point 10), Yeoncheon-eup, Yeoncheon-gun, Gyeonggi Province; FP 60 (Firing Point 60), Jeongok-eup, Yeoncheon-gun, Gyeonggi Province; Chaparral Training Area, and LTA 130 (Local Training Area 130), Misan-myeon, Yeoncheon-gun, Gyeonggi Province; and MPRC (Multipurpose Range Complex=Rodriguez Range), Youngjung-myeon, Pocheon-gun, Gyeonggi Province.

agents can provide a better understanding of disease maintenance cycles and possibly a means of predicting disease emergence events (Nieto et al., 2007).

Flea species and host-parasite relationships were identified to determine the prevalence of the flea-borne pathogen *Rickettsia typhi* in small mammals and their associated flea species in the ROK. The seasonal prevalence of fleas collected from wild-caught small mammals at selected military training sites in northern Gyeonggi province near the Demilitarized Zone (DMZ) and associated flea-borne pathogens are reported.

#### MATERIALS AND METHODS

Fleas were collected from wild rodents and insectivores that were live-trapped at US- and ROK-operated military training sites near the

DMZ (Fig. 1). Seasonal surveys were conducted during the spring (March–April), summer (June), fall (late August–September), and winter (late November–December) of 2003–04. Sherman traps (7.7×9×23 cm aluminum collapsible live-traps, H. B. Sherman, Tallahassee, Florida, USA) were baited with peanut butter placed between two saltine crackers and were set out during daylight hours and collected the following morning. Nonabsorbent cotton balls were placed in each trap during the winter and spring trapping periods, as nighttime temperatures often fell below 0 C, so trapped animals would retain heat until processed. Fleas were identified to species using conventional taxonomic keys; fleas were placed in 1.0 ml cryovials, and stored individually at –70 C until assayed for selected pathogens (Hopkins and Rothschild, 1953, 1956; Hong, 1994). Flea infestation rates, flea indices, and minimum field infection rates (MFIR) were determined by the following formulas: Flea Infestation Rate=Number of captured small mammals with fleas/Total num-

TABLE 1. Number of fleas collected, flea infestation rates, and flea indices for small mammals captured at 10 US-operated and Republic of Korea (ROK)-operated military training sites and one US military installation, using collapsible live-capture Sherman traps, northern Gyeonggi Province, ROK, 2003–04.

Host species	Total no. captured	No. with fleas	Infestation rate (%) <sup>a</sup>	No. fleas	Mean no. fleas <sup>b</sup>	Flea index (FI) <sup>c</sup>
<i>Apodemus agrarius</i>	2,439	484	19.8	834	1.7	0.34
<i>Mus musculus</i>	79	10	12.7	13	1.3	0.17
<i>Micromys minutus</i>	13	1	7.7	1	1.0	0.08
<i>Rattus norvegicus</i>	9	1	11.1	3	3.0	0.33
<i>Microtus fortis</i>	59	12	20.3	24	2.0	0.41
<i>Tscherskia triton</i>	13	1	7.7	1	1.0	0.08
<i>Myodes regulus</i>	16	5	31.3	11	2.2	0.69
<i>Crocidura lasiura</i>	114	24	21.1	61	2.5	0.54
TOTAL	2,742	538	19.6	948	1.7	0.35

<sup>a</sup> Percent of small mammals captured with fleas.

<sup>b</sup> Average number of fleas per infested animal for each species.

<sup>c</sup> Flea index (FI)=Number of fleas/total number of trapped rodents.

ber of small mammals captured; Flea Index=Number of fleas collected from small mammals/Number of small mammals captured; Minimum field infection rate (MFIR)=Number of positive pools of fleas/Total number of fleas.

For *R. typhi* testing, DNA was extracted from 220 pools (654 fleas, 2–5 fleas/pool). Fleas were pooled according to collection date and site, species, and host animal species. Fleas were homogenized mechanically using a sterile manual homogenizer (General Biosystem, Seoul, Korea), followed by DNA extraction performed with DNeasy<sup>®</sup> tissue kits (Qiagen, Hilden, Germany) per manufacture instructions.

Primers (primer 1, 5'-GCTCTTGCAACT-TCTATGTT-3' and primer 2, 5'-CATTGTT-CGTCAGGTTGCCG-3') were used for detection of *R. typhi* 17 kDa antigenic protein gene fragment by polymerase chain reaction (PCR) amplification as described by Webb et al. (1990). For PCR, cycle temperatures and times were as follows: denaturing at 94 C for 30 sec, annealing at 57 C for 2 min, and extension at 70 C for 2 min; 35 cycles were used. Positive PCR products were sequenced and identified from GenBank database (Altschul et al., 1990). Positive control DNA of *R. typhi* was supplied by the Division of Zoonosis, Center for Immunology and Pathology, Korea National Institute of Health. DNA samples from negative wild rodent and fleas were used for negative controls.

## RESULTS

During 2003 and 2004, 2,742 small mammals belonging to seven genera of rodents and one genus of insectivore were

collected from 10 US- and ROK-operated military training sites and one US military installation (Table 1 and Fig 1). *Apodemus agrarius* (striped field mouse) accounted for 88.9% (2,439/2,742) of the small mammals captured, followed by *Crocidura lasiura* ( $n=114$ , 4.2%), *Mus musculus* ( $n=79$ , 2.9%), *Microtus fortis* ( $n=59$ , 2.2%), *Myodes regulus* ( $n=16$ , 0.6%), *Micromys minutus* ( $n=13$ , 0.5%), *Tscherskia triton* ( $n=13$ , 0.5%), and *Rattus norvegicus* ( $n=9$ , 0.3%) (Table 1).

In total, 948 fleas (563 females and 385 males) were collected from 538/2,742 (19.6%) small mammal hosts during 2003–04 (Table 2). Flea infestation rates ranged from 7.7% (*M. minutus* and *T. triton*) to 31.3% (*M. regulus*) (Table 1). The number of fleas recovered from infested small mammals ranged from 1 to 13, while the mean number of fleas recovered from “infested” small mammals was 1.7, ranging from a low of 1.0 (*M. minutus* and *T. triton*) to a high of 3.0 (*R. norvegicus*) for different species (Table 1). The overall flea index for all small mammals for both years was 0.35. Among the small mammals captured, the flea index was highest for *M. regulus* (0.69), followed by *C. lasiura* (0.54), *A. agrarius* (0.34), *R. norvegicus* (0.33), *M. musculus* (0.17), *M. minutus* (0.08), and *T. triton* (0.08).

The overall flea infestation rates were

TABLE 2. Seasonal abundance of fleas and flea indices for small mammals captured at 10 US-operated and Republic of Korea (ROK)-operated military training sites and one US military installation, using collapsible live-capture Sherman traps, northern Gyeonggi Province, ROK, 2003–04.

Collection sites <sup>a</sup>	No. small mammals	No. (%) infested	No. fleas	Flea index <sup>b</sup>	No. fleas collected (flea infestation rate)			
					Spring (n=672) <sup>c</sup>	Summer (n=738) <sup>c</sup>	Fall (n=527) <sup>c</sup>	Winter (n=805) <sup>c</sup>
Warrior Base Complex	112	20 (17.9)	33	0.59	7 (21.2)	2 (6.1)	0	24 (72.7)
Monkey #7 Training Area	274	59 (21.5)	95	0.35	16 (16.8)	32 (33.7)	6 (6.3)	41 (43.2)
Story Range	223	45 (20.2)	90	0.40	28 (31.1)	0	5 (5.6)	57 (63.3)
Dagmar North	867	161 (18.6)	249	0.29	50 (20.1)	66 (26.5)	18 (7.2)	115 (46.2)
Firing Point 10	279	66 (23.7)	101	0.36	38 (37.6)	15 (14.9)	10 (9.9)	38 (37.6)
Firing Point 60	388	79 (20.4)	134	0.35	37 (27.6)	40 (29.9)	13 (9.7)	44 (32.8)
Chaparral Training Area	59	7 (11.9)	9	0.15	5 (55.6)	0	2 (22.2)	2 (22.2)
Local Training Area 130	101	26 (25.7)	54	0.53	13 (24.1)	0	0	41 (75.9)
MPRC (=Rodriguez Range)	439	75 (17.1)	183	0.42	23 (12.6)	19 (10.4)	5 (2.7)	136 (74.3)
TOTAL	2,742	538 (19.6)	948	0.35	217 (22.9)	174 (18.4)	59 (6.2)	498 (52.5)

<sup>a</sup> Warrior Base Complex (North Carolina Range, Jangdan-myeon, Paju-si, Gyeonggi Province; Camp Bonifas, and Warrior Base, Gunnae-myeon, Paju-si, Gyeonggi Province); Monkey #7 and Story Range: Jindong-myeon, Paju-si, Gyeonggi Province; Dagmar North: Jeokseong-myeon, Paju-si, Gyeonggi Province; Firing Point 10: Yeoncheon-eup, Yeoncheon-gun, Gyeonggi Province; Firing Point 60: Jeongok-eup, Yeoncheon-gun, Gyeonggi Province; Chaparral Training Area, and Local Training Area 130: Misan-myeon, Yeoncheon-gun, Gyeonggi Province; and MPRC (Multipurpose Range Complex=Rodriguez Range): Youngjung-myeon, Pocheon-gun, Gyeonggi Province.

<sup>b</sup> Flea index=Number of fleas/total number of trapped rodents.

<sup>c</sup> Number of small mammals captured.

lowest during the fall (6.2%) and highest during winter season (52.5%) (Table 2). The area with the highest number of rodents infested with fleas was Local Training Area (LTA) 130 (25.7%), even though no flea-infested small mammals were collected during the summer and fall trapping seasons. Flea indices (FI) ranged from 0.15 to 0.59 for the sites surveyed (Table 2).

The most common flea species recovered was *Ctenophthalmus congeneroides* (51.3%), followed by *Stenoponia sidimi* (42.6%), *Rhadinopsylla insolita* (5.5%), *Neopsylla bidentatiformis* (0.4%), *Rhadinopsylla concava* (0.1%), and *Doratopsylla coreana* (0.1%) (Table 3). *Rhadinopsylla concava* and *D. coreana*, while infrequently collected, were only collected from the insectivore, *C. lasiura*. *Ctenophthalmus congeneroides* specimens were collected during all trapping seasons, and high populations were recorded in the spring through fall (Fig. 2). Conversely, *S. sidimi* individuals were collected from small mammals during all trapping periods but

were most commonly collected during the winter trapping period (Fig. 2). *Rhadinopsylla insolita* and *S. sidimi* specimens were collected only during the spring and winter trapping periods, while *R. concava* and *D. coreana* were collected only in the winter.

Seven pools from 220 pools, out of a total of 654 fleas, were positive for *R. typhi* (3.2%), and MFIR=1.1%, using species-specific PCR techniques on pooled DNA samples (Table 4). All seven positive pools were from fleas collected from *A. agrarius* during the March and December trapping periods: four *C. congeneroides* from Firing Points 10 and 60 (Yeoncheon-gun, Gyeonggi Province), one *R. insolita* from Story Range (Jindong-myeon, Paju-si, Gyeonggi Province), one *C. congeneroides* from Dagmar North Training Area (Jeokseong-myeon, Paju-si, Gyeonggi Province), and one *R. insolita* from LTA 130 (Misan-myeon, Yeoncheon-gun, Gyeonggi Province; Fig. 1). While all positive pools were collected from *A. agrarius*, all *A. agrarius* from the seven positive pools were negative by immunofluorescence

TABLE 3. Number (%) of each species of flea recovered from selected small mammal hosts collected at 10 US-operated and Republic of Korea (ROK)-operated military training sites and one US military installation, northern Gyeonggi Province, ROK, 2003–04.

Fleas host species	<i>Stenoponia sidimi</i>	<i>Rhadinopsylla insolita</i>	<i>Rhadinopsylla concava</i>	<i>Ctenophthalmus congeneroides</i>	<i>Neopsylla bidentatiformis</i>	<i>Doratomyia coreana</i>	TOTAL (%)
<i>Apodemus agrarius</i>	375 (45.0)	29 (3.5)	0	427 (51.2)	3 (3.6)	0	834 (88.0)
<i>Mus musculus</i>	1 (7.7)	0	0	12 (92.3)	0	0	13 (1.4)
<i>Micromys minutus</i>	0	0	0	1 (100)	0	0	1 (0.1)
<i>Rattus norvegicus</i>	0	0	0	3 (100)	0	0	3 (0.3)
<i>Microtus fortis</i>	4 (16.7)	4 (16.7)	0	15 (62.5)	1 (4.2)	0	24 (2.5)
<i>Tscherskia triton</i>	0	0	0	1 (100)	0	0	1 (0.1)
<i>Myodes regulus</i>	1 (9.1)	0	0	10 (90.9)	0	0	11 (1.2)
<i>Crocidura lasiura</i>	23 (37.7)	19 (31.1)	1 (1.6)	17 (27.9)	0	1 (1.6)	61 (6.4)
TOTAL (%)	404 (42.6)	52 (5.5)	1 (0.1)	486 (51.3)	4 (0.4)	1 (0.1)	948 (100.0)

antibody (IFA) tests for murine typhus. Oligonucleotide sequences of the PCR products (EU532434 for *R. insolita*, EU532435 for *C. congeneroides*) were identical to *R. typhi* 17 kDa genus-common antigen gene (GenBank accession number, AY867871; Reeves et al., 2005).

## DISCUSSION

Fleas are very mobile and will often abandon their natural host(s), especially

after death of the host; these fleas will readily utilize other hosts, including humans (Azad et al., 1977). Flea infestations in this study were generally higher during the midwinter months and were similar to those observed by Walton and Hong (1976). An exception was *C. congeneroides*, which were collected more frequently during the spring, summer, and fall trapping periods. The biology of these species is not well known in Korea and may reflect host and/or ectoparasite reproductive seasonal differences.

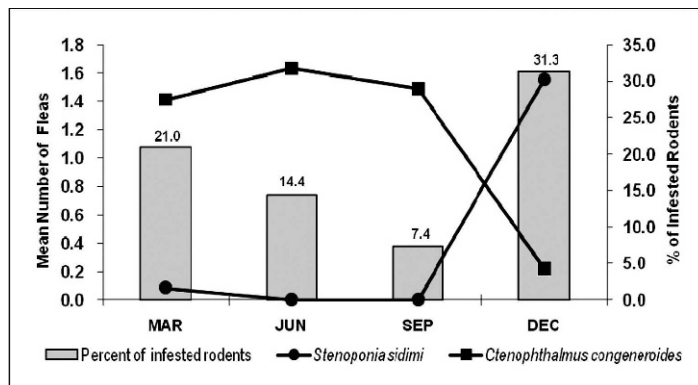


FIGURE 2. Mean number of fleas and percent of infested small mammals captured at 10 US-operated and Republic of Korea (ROK)-operated military training sites and one US military installation, northern Gyeonggi Province, ROK, 2003–04.

TABLE 4. *Rickettsia typhi* identified by PCR in selected flea species from *Apodemus agrarius* collected at five training sites, Republic of Korea, 2003–04.

Species	No. fleas	No. of pooled DNA <sup>a</sup>	No. of PCR positive (MFIR <sup>b</sup> ) for <i>Rickettsia typhi</i>
<i>Stenoponia sidimi</i>	287	81	0
<i>Ctenophthalmus congesteroides</i>	317	112	5 (1.6)
<i>Rhadinopsylla insolita</i>	48	25	2 (4.2)
<i>Neopsylla bidentatiformis</i>	2	2	0
Total	654	220 <sup>a</sup>	7 (1.1)

<sup>a</sup> 2–5 fleas per pool (total 654 fleas).

<sup>b</sup> MFIR (minimum field infection rates)=Number positive pools/Total number fleas assayed.

The PCR techniques used in our study for detection of *R. typhi* (Webb et al., 1990) have been used for identifying *Rickettsia* pathogens in the cat flea, *Ctenocephalides felis*, from various countries throughout the world, including Cyprus (Psaroulaki et al., 2006), Mexico (Zavala-Castro et al., 2005), Peru (Blair et al., 2004), Afghanistan (Marie et al., 2006), USA (Reeves et al., 2005), and Israel (Bauer et al., 2006). Positive pools found in this study were observed from fleas taken only from *A. agrarius* that were negative by IFA for *R. typhi*. While fleas tested in this study had blood meals and were removed from *R. typhi*-negative hosts, this does not preclude them from having taken a recent blood meal from a *R. typhi*-positive host.

Until now, there have been no reports on the epidemiology of flea-borne pathogens and reservoir host/vector associations in the ROK, compared with numerous reports throughout the world. This study has enabled us to provide further information on the epidemiology of potential flea-associated pathogens in the ROK. Further studies are required for a better understanding of potential emerging zoonotic flea-borne diseases in the ROK, which will enable health professionals to be more responsive to environmental changes and human activities that may impact on the health of civilian and military populations. It is imperative to continue the efforts to identify flea-borne pathogens to further illuminate the extent

and potential public health significance of these ectoparasitic disease agents.

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