Comparison of Dragging and Sweeping Methods for Collecting Ticks and Determining Their Seasonal Distributions for Various Habitats, Gyeonggi Province, Republic of Korea

SUNG TAE CHONG,[1] HEUNG CHUL KIM,[1] IN-YONG LEE,[2] THOMAS M. KOLLARS, JR.,[3] ALFREDO R. SANCHO,[4] WILLIAM J. SAMES,[5] AND TERRY A. KLEIN[6]

J. Med. Entomol. 50(3): 611–618 (2013); DOI: http://dx.doi.org/10.1603/ME12032

ABSTRACT As part of the 65th Medical Brigade tick-borne disease surveillance program to determine the abundance, geographical and seasonal distributions, and tick-borne pathogens present in the Republic of Korea, dragging and sweeping methods were compared to determine their efficiency for collecting ticks in grass and deciduous, conifer, and mixed forest habitats at military training sites and privately owned lands in northern Gyeonggi Province near the demilitarized zone from April–October, 2004–2005. Three species of Ixodid ticks, *Haemaphysalis longicornis*, *Haemaphysalis flava*, and *Ixodes nipponensis*, were collected. Overall, *H. longicornis* adults and nymphs were most frequently collected from grass and deciduous forest habitats, accounting for 98.2 and 66.2%, respectively, of all ticks collected. *H. flava* adults and nymphs were most frequently collected from conifer and mixed forests, accounting for 81.6, and 77.8%, respectively, of all ticks collected. *I. nipponensis* adults and nymphs accounted for 9.3% of all ticks collected from mixed forests, were less commonly collected from deciduous (4.1%) and conifer (4.1%) forests, and infrequently collected from grass habitats (0.9%). Overall, there were no significant differences between dragging and sweeping methods for the three species when the areas sampled were similar (sweeping = 2 × the area over the same transect). Adults and nymphs of *H. longicornis* were most commonly collected from April–August, while those of *H. flava* and *I. nipponensis* were most commonly collected during April–July and again during October. Larvae of all three species were most frequently observed from July–September.

KEY WORDS tick, sweeping, dragging, habitats, seasonal prevalence

Tick-borne disease surveillance must include effective and efficient methods for collecting ticks to estimate abundance and geographical distributions and the prevalence of tick-borne zoonoses of medical and veterinary importance (Takada et al. 1993, Mahara 1997, Murphy et al. 1998, Chiba et al. 1999, Alekseev et al. 2001, Fournier et al. 2002, Ishikura et al. 2002). Tick-borne pathogens affecting human and animal health in the Republic of Korea (ROK), include members of the spotted fever group (SFG) rickettsiae (Lee et al. 2003, Jang et al. 2004), *Ehrlichia* and *Anaplasma* spp. (Chae et al. 2003), *Bartonella* spp. (Kim et al. 2005), *Borrelia burgdorferi* (Park et al. 1992, Kee et al. 1994), and tick-borne encephalitis virus (western subtype) (Kim et al. 2008, 2009b; Ko et al. 2010). Ticks collected from birds (bird banding surveys) and small-large animals (surveys of feral and domestic animals) serve to associate host, pathogen, and tick relationships (Kim et al. 2009a, Sames et al. 2010, Kim et al. 2011). Previous studies have shown disparities among tick sampling techniques for collecting ticks, as some species are more frequently collected from animal hosts, while few are collected when dragging or sweeping for ticks (Falco and Fish 1992, Carroll and Schmidtmann 1992, Terassini et al. 2010). Alternatively, some ixodid species may be efficiently collected from vegetation using dragging or sweeping techniques. The direct collection of ticks from animal and bird hosts is often impractical and less efficient as they require their live capture (Falco and Fish 1992). Therefore, other methods, that is, dragging and sweeping for ticks should be considered to determine their geographical and habitat distributions, relative population densities, and

The opinions and assertions contained herein are those of the authors and are not to be construed as official or reflecting the views of the Department of the Army or the Department of Defense.

[1] 5th Medical Detachment, 168th Multifunctional Medical Battalion, 65th Medical Brigade, Unit #15247, APO AP 96205–5247.

[2] Department of Environmental Medical Biology, Yonsei University College of Medicine, 120[752, Seoul, Korea.

[3] Medical Section, 415th Chemical Brigade, 814 Perimeter Road, Greenville, SC 29605.

[4] U.S. Public Health Service Commissioned Corps, 8904 Darnestown Road, Rockville, MD 20850.

[5] Armed Forces Pest Management Board, WRAMC-Forest Glen Annex, Bldg. 172, 6900 Georgia Ave., NW, Washington, DC 20307–5001.

[6] Corresponding author, Public Health Command Region-Pacific/65th Medical Brigade/US Army MEDDAC-Korea, Unit #15281, APO AP 96205–5281 (e-mail: terry.klein@us.army.mil).

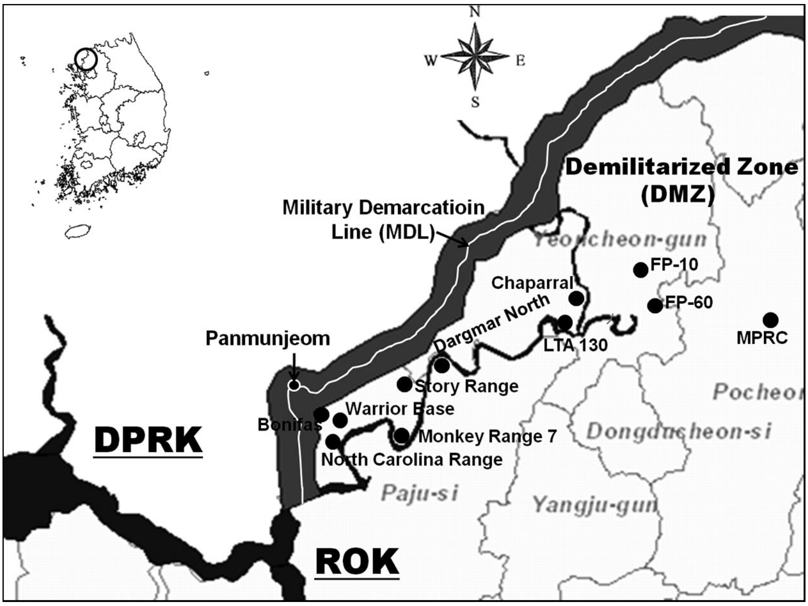

Fig. 1. Geographical location of tick collection sites at U.S. installations and U.S./ROK operated training sites conducted near the demilitarized zone, Gyeonggi Province, April–October 2004–2005 (North Carolina Range [37° 53′ 04.29″ N, 126° 43′ 20.22″ E], Camp Bonifas [37° 55′ 51.64″ N, 126° 43′ 13.90″ E], Warrior Base [37° 55′ 15.02″ N, 126° 44′ 45.04″ E], Monkey Range 7 [37° 53′ 44.30″ N, 126° 48′ 08.61″ E], Story Range [37° 57′ 14.85″ N, 126° 48′ 17.12″ E], Dagmar North [37° 58′ 02.32″ N, 126° 50′ 27.00″ E], Local Training Area 130 [LTA 130] [38° 01′ 06.0.98″ N, 126° 59′ 22.15″ E], Firing Point-10 [FP-10] [38° 04′ 41.70″ N, 127° 04′ 37.00″ E], Firing Point-60 [FP-60] [38° 02′ 55.34″ N, 127° 06′ 18.18″ E], Chaparral Training Area [38° 02′ 40.05″ N, 126° 58′ 55.03″ E], Rodriguez Range [MPRC] [38° 01′ 28.36″ N, 127° 13′ 10.12″ E]).

presence and distribution of tick-borne pathogens. In addition, assays of unfed questing larval ticks can be used to determine the transovarial transmission potential. These data form the background for the development of vector-borne threat assessments and mitigation strategies to reduce risks associated with tick bites and transmission of tick-borne pathogens, in addition to medical awareness for rapid diagnosis and treatment of patients to reduce morbidity and mortality.

The primary purpose of the current study was to compare two widely used sampling methods, dragging and sweeping, for the collection of ticks and to integrate these methods as part of a comprehensive 65th Medical Brigade tick-borne disease surveillance program in the ROK. These data provide: 1) a measure of potential human exposure to ticks for selected habitats, 2) identify tick borne pathogens and prevalence of infections, 3) identify tick-borne medical threats for military and civilian populations training or performing recreational activities in unmanaged habitats infested with ticks (reported elsewhere), and 4) the potential impact (i.e., economic losses) of tick-borne diseases among wildlife and domestic animals.

Materials and Methods

Habitats. As part of the 65th Medical Brigade tick-borne disease surveillance program, tick collections were conducted at U.S. military training sites and privately owned properties located near the demilitarized zone (DMZ) that separates North and South Korea, northern Gyeonggi Province, from April–October, 2004–2005 (O'Guinn et al. 2008; Kim et al. 2010a,b, 2011; Klein et al. 2012a, b) (Fig. 1). Environmental habitats were selected that included: 1) grasses and other herbaceous vegetation (i.e., *Humulus japonicas* and *Artemisia* spp.) <0.5 m and 2) deciduous, 3) conifer, and 4) mixed forests where U.S. military personnel conducted field exercises and were exposed to ticks and at nearby privately owned properties (O'Guinn et al. 2008, Kim et al. 2009a, Sames et al. 2009). Forested areas often consisted of pure stands of planted groves of pine, fir, larch, chestnut, and oak trees or mixed forests planted as part of a national tree planting policy in the 1960s (Ko et al. 2010). Forest ground cover consisted of dead leaves and needles, with limited understory with various types of grasses, herbaceous vegetation, and shrubs. Only grasses <0.5 m in height were surveyed because previous surveys of taller grasses yielded few ticks. While some sites were routinely surveyed at monthly intervals, others were surveyed less frequently because of limited or restricted access at the time of the survey.

Dragging and Sweeping Methods. Sampling was conducted monthly from April through October when ticks were most active. Tick drags consisted of a 1.5 m long × 1.0 m wide flannel cloth with the leading edge attached to a wooden dowel (1.1 m long, 2.0 cm diameter) and a small diameter 4-m long nylon rope attached to each end of the wooden dowel, as previously described by Daniels et al. (1998) and Scoles et al. (2001). Sweeping vegetation for ticks was conducted as described by Carroll and Schmidtmann (1992), except that the 1.5 m (length) × 1 m (wide) flannel cloth was attached to an aluminum pole bent at a 45° angle 1-m from the end where the leading edge of the flannel cloth was attached. The flannel cloth was dragged behind the collector walking at a slow pace over vegetation or ground litter, or was swept from side to side parallel to the ground over vegetation in front of the collector as the collector slowly walked forward (Falco and Fish 1992, Carroll and Schmidtmann 1992, Terassini et al. 2010). Questing ticks on the vegetation and ground litter attached to the cloth and were periodically removed (5 m intervals) by the collector by laying the cloth, bottom side up, on the ground, removing attached ticks using a fine forceps, and then alternately examining the top side for ticks before continuing. Up to 20 nymphs and adults were placed in 2-ml cryovials, numbered, and then secured in a Styrofoam cooler. Because of the large numbers of larvae, a portion of each newly hatched cluster was collected and up to 100 placed in 2-ml cryovials containing 100% ethanol. This was repeated until each collector traveled a distance of ≈100 m.

Generally four 100 m long linear transects were surveyed concurrently. Each transect (replicate) was spaced at least 10 m from the habitat edge to reduce border effect. Paired transects were separated by at least 10 m, alternating sweeping and dragging, while the collectors maintained a similar pace and line-of-site to ensure that the 10 m distance separating transect lanes was maintained. Collectors were alternated between dragging and sweeping to reduce collector bias. Where habitats were limited, only two transects, paired dragging and sweeping, were conducted. The area surveyed by dragging consisted of a 1-m wide cloth × 100-m long transect (100 m^2), while the sweeping techniques covered an estimated area of 200 m^2 (2-m side to side motion × 100-m long transect).

After each collection period, ticks were transported to the fifth Medical Detachment, Yongsan U.S. Army Garrison, Seoul, Korea, where they were identified to species and developmental stage under a dissecting microscope using standard keys and current nomenclature (Yamaguti et al. 1971, Robbins and Keirans 1992) and maintained at −70°C to be later assayed for rickettsial and other tick-borne pathogens (Kim et al. 2005, 2006; Lee et al. 2005; Park et al. 2005).

Data Analysis. An electronic data sheet (Microsoft Excel) was prepared for each collection site and later updated to include species and stage of development for data management. All adults and nymphs were collected, but only a sample of larvae were collected (up to 50/cluster/5 m intervals) because of large numbers often encountered in heavily infested areas and thus were excluded from analysis to reduce bias. Area surveyed per transect by dragging was estimated at 100 m^2 (1-m wide flannel cloth/100 m distance). The area surveyed by sweeping the flannel cloth from side-to-side was estimated at 200 m^2 (2-m wide sweeping motion/100 m distance). Comparative analysis between mean numbers of nymphs and adults collected for each species and habitat surveyed was conducted using Tukey pairwise comparison, one way analysis of variance (ANOVA). The paired *t*-test was used for direct comparisons between dragging and sweeping methods for each of the habitats and tick species. Similarly, the paired *t*-test was used for comparisons between the numbers of ticks collected for the area of each transect sampled using [dragging, [number of ticks/100m^2; sweeping, (number of ticks/200m^2)/2]].

Results

Dragging and Sweeping. In total, 136 paired replicate 100 m transects (grand total 272 transects), using both dragging and sweeping methods, were surveyed for ticks. Habitats that were surveyed were characterized as: 1) grasses and herbaceous vegetation <0.5 m (49 paired replicates) and 2) deciduous (34 paired replicates), 3) conifer (27 paired replicates), and 4) mixed (26 paired replicates) forests (Tables 1 and 2). In total, 1,033 adults and 6,394 nymphs belonging to two genera and three species, *H. longicornis* (865 adults; 5,282 nymphs), *H. flava* (133 adults; 993 nymphs), and *I. nipponensis* (35 adults; 119 nymphs) were collected (Table 1). An additional 12,394 larvae were collected belonging to *H. longicornis* (8,873), *H. flava* (2,763), and *I. nipponensis* (758), but were excluded from analysis. Overall, *H. longicornis* adults and nymphs accounted for 82.8% (6,147) of the three species collected from all habitats, followed by *H. flava* 15.1% (1,126) and *I. nipponensis* 2.1% (154) (Table 1). *H. longicornis* adults and nymphs accounted for 98.2% (5,427/5,524) and 66.2% (580/876) from grass and deciduous forest habitats, respectively, while *H. flava* accounted for only 0.9 and 29.7%, respectively, and *I. nipponensis* accounted for only 0.9 and 4.1%, respectively. *H. flava* adults and nymphs were more frequently collected from conifer forests (81.6%, 433/531) and mixed forests (77.8%, 386/496) than *H. longicornis* (14.3 and 4.1%, respectively) or *I. nipponensis* (4.1 and 9.3%, respectively).

Significantly more *H. longicornis* adults and nymphs were collected for both dragging and sweeping techniques combined (ANOVA; $F = 10.88$; df = 3; $P < 0.001$) (mean number 55.4) from grass habitats, while there was no significant difference in the mean numbers collected from deciduous (8.5), mixed (1.4), and conifer (1.2) forest habitats (Table 2). Alternatively, significantly more *H. flava* adults and nymphs were collected from conifer (8.0) and mixed (7.4) forest habitats ($F = 15.98$; df = 3; $P < 0.001$), while lower mean numbers were collected from deciduous forest (3.8) and grass (0.5) habitats (Table 2). *I. nipponensis* only accounted for 2.1% of the total number of ticks collected by both dragging and sweeping (Table 1). While significantly more *I. nipponensis* nymphs were collected from mixed forests (mean 0.8) ($F = 2.799$; df = 3; $P = 0.04$), there were no significant differences

Table 1. Number (mean) of adult and nymph ixodid ticks and percent collected, by species and habitat, while dragging and sweeping vegetation at United States and Korean operated military training sites and private lands located near the DMZ from April through Oct. 2004–2005, Gyeonggi Province, Republic of Korea

Habitat (no. replicates)[a]	Species	Drag number (mean)[b]		Sweep number (mean)[b]		Total (mean)[c] no. nymphs and adults	Percent, by habitat[d]
		Nymph	Adult	Nymph	Adult		
Grass (n = 49)	*H. flava*	15 (0.3)	5 (0.1)	24 (0.5)	3 (0.1)	47 (1.0)	0.9%
	H. longicornis	2,041 (41.7)	261 (5.3)	2,747 (57.2)	378 (7.7)	5,427 (110.8)	98.2%
	I. nipponensis	13 (0.3)	12 (0.1)	14 (0.3)	11 (0.2)	50 (1.0)	0.9%
Deciduous Forest (n = 34)	*H. flava*	83 (2.4)	10 (0.3)	149 (4.4)	18 (0.5)	260 (7.6)	29.7%
	H. longicornis	193 (5.7)	82 (2.4)	197 (5.8)	108 (3.2)	580 (17.1)	66.2%
	I. nipponensis	7 (0.2)	2 (0.1)	25 (0.7)	2 (0.1)	36 (1.1)	4.1%
Conifer Forest (n = 27)	*H. flava*	115 (4.3)	18 (0.7)	268 (9.9)	32 (1.2)	433 (16.0)	81.6%
	H. longicornis	17 (0.6)	6 (0.2)	42 (1.6)	11 (0.4)	76 (2.8)	14.3%
	I. nipponensis	2 (0.1)	2 (0.1)	16 (0.6)	2 (0.1)	22 (0.8)	4.1%
Mixed Forest (n = 26)	*H. flava*	157 (6.0)	19 (0.7)	182 (7.0)	28 (1.1)	386 (14.8)	77.8%
	H. longicornis	18 (0.7)	8 (0.3)	27 (1.0)	11 (0.4)	64 (2.5)	12.9%
	I. nipponensis	15 (0.6)	4 (0.2)	27 (1.0)	0 (0)	46 (1.7)	9.3%
Subtotal (n = 136)	*H. flava*	370 (2.7)	52 (0.4)	623 (4.6)	81 (0.6)	1,126 (8.3)	15.1%
	H. longicornis	2,269 (16.7)	357 (2.6)	3,013 (22.2)	508 (3.7)	6,147 (45.2)	82.8%
	I. nipponensis	37 (0.3)	20 (0.1)	82 (0.6)	15 (0.1)	154 (1.1)	2.1%
Total		2,676	429	3,718	604	7,427	

[a] No. of paired replicates (dragging and sweeping) for each habitat.
[b] No. of adults and nymphs collected/Number of replicates.
[c] No. of adults and nymphs collected by dragging and sweeping/Total no. of replicates.
[d] No. Percent of both adults and nymphs collected, by species, for each of the habitats surveyed.

for adults and nymphs combined from all habitats (grasses, 0.5; deciduous forests, 0.5; conifer forests, 0.4; and mixed forests, 0.9) sampled (F = 1.44; df = 3; P = 0.23) (Table 2).

The overall mean number of *H. longicornis* collected by dragging (19.31) was not significantly different when compared with sweeping over 200 m^2 (25.89) (P = 0.200) or 100 m^2 (12.94) (P = 0.140) (Table 3). The overall mean numbers of *H. flava* adults and nymphs collected from grass and forest habitats by sweeping (200 m^2; 5.18) were significantly higher than for dragging (100 m^2; 3.10) over each transect ($P <$ 0.001) (Table 3). When equivalent areas (number of ticks collected by sweeping/2) sampled for each method were considered, the mean number of ticks collected by sweeping (2.59), while less than for dragging (3.10) for the paired transects, was not significantly different (P = 0.111) (Table 3). While significantly more *I. nipponensis* were collected by sweeping (0.71) than dragging (0.42) over all habitats (P = 0.016), there were no significant differences when equal areas were sampled by dragging (0.42) or sweeping (0.36) (P = 0.429) (Table 3). Similarly, overall both *H. flava* and *I. nipponensis* adults and nymphs were collected more frequently by sweeping in deciduous and conifer forests, but when the same area sampled was considered, there were no significant differences (Table 3).

Seasonal Distribution. The seasonal distributions for adults, nymphs, and larvae of *H. longicornis*, *H. flava*, and *I. nipponensis* collected by both dragging and sweeping are shown in Fig. 2. In general, for all habitats, *H. longicornis* adults were collected more frequently from June–August (Fig. 2A). Nymphs were most prevalent from April–June, while larvae were collected mostly during August–September (Fig. 2A,D). *H. flava* adult populations peaked in May, with lower numbers collected during for the remaining months (Fig. 2B). *H. flava* nymph populations were bimodal, with increased numbers collected during May and October and lower numbers collected during the remaining months surveyed. The increased num-

Table 2. Total no. of ticks collected by both methods (dragging and sweeping) and comparison of the mean numbers of adults and nymphs, by species and habitats, at US and Korean operated military training sites and private lands located near the demilitarized zone (DMZ) from April through Oct. 2004–2005, Gyeonggi Province, Republic of Korea

Habitat	No. replicates[a]	*Haemaphysalis longicornis*			*Haemaphysalis flava*			*Ixodes nipponensis*		
		No. nymphs (mean)[b,c]	No. adults (mean)[b,c]	Total no. (mean)[b,c]	No. nymphs (mean)[b,c]	No. adults (mean)[b,c]	Total no. (mean)[b,c]	No. nymphs (mean)[b,c]	No. adults (mean)[b,c]	Total no. (mean)[b,c]
Grass	98	4,788 (48.9)[a]	639 (6.5)[a]	5,427 (55.4)[a]	39 (0.4)[a]	8 (0.1)[a]	47 (0.5)[a]	27 (0.3)[a]	23 (0.2)[a]	50 (0.5)[a]
Deciduous	68	390 (5.7)[b]	190 (2.8)[b]	580 (8.5)[b]	232 (3.4)[b]	28 (0.4)[b]	260 (3.8)[b]	32 (0.5)[a,b]	4 (0.1)[a]	36 (0.5)[a]
Conifer	54	59 (1.1)[b]	17 (0.3)[b]	76 (1.4)[b]	383 (7.1)[c]	50 (0.9)[c]	433 (8.0)[c]	18 (0.3)[a]	4 (0.1)[a]	22 (0.4)[a]
Mixed	52	45 (0.9)[b]	19 (0.4)[b]	64 (1.2)[b]	339 (6.5)[c]	47 (0.9)[c]	386 (7.4)[c]	42 (0.8)[b]	4 (0.1)[a]	46 (0.9)[a]
Total	272	5,282 (19.4)	865 (3.2)	6,147 (22.6)	993 (3.7)	133 (0.5)	1,126 (4.1)	119 (0.4)	35 (0.1)	154 (0.6)

[a] Total no. of 100 m transects surveyed by dragging and sweeping for ticks.
[b] Mean no. of ticks collected/100 m transect for both dragging and sweeping methods.
[c] Like letters indicate that the mean no. of ticks collected, by stage of development and species for each of the habitats, are not significantly different (ANOVA using Tukey pairwise comparison, $P <$ 0.05).

Table 3. Total and mean no. of adult and nymph and ticks by dragging and sweeping vegetation over an approximate distance of 100 m for four habitats at military training sites and private lands located near the DMZ, Gyeonggi Province, Republic of Korea, from April–Oct. 2004–2005

Habitat	No. replicates[a]	Species	Collection method: Drag – No. collected	Drag – Mean no./100 m² [b]	Sweep – No. collected	Sweep – Mean no./200 m² [c]		Sweep – Mean no./100 m² [d]	
Grass	49	*H. flava*	20	0.41	27	0.55	($P = 0.322$)	0.28	($P = 0.271$)
		H. longicornis	2,302	46.98	3,125	63.78	($P = 0.239$)	31.89	($P = 0.206$)
		I. nipponensis	25	0.51	25	0.51	($P = 1.000$)	0.26	($P = 0.091$)
Deciduous Forest	34	*H. flava*	93	2.74	167	4.91	($P = 0.008$)[e]	2.46	($P = 0.550$)
		H. longicornis	275	8.09	305	8.97	($P = 0.668$)	4.49	($P = 0.081$)
		I. nipponensis	9	0.26	27	0.79	($P = 0.007$)[e]	0.40	($P = 0.141$)
Conifer Forest	27	*H. flava*	133	4.93	300	11.11	($P = 0.005$)[e]	5.56	($P = 0.422$)
		H. longicornis	23	0.85	53	1.96	($P = 0.524$)	0.98	($P = 0.509$)
		I. nipponensis	4	0.15	18	0.67	($P = 0.017$)[e]	0.33	($P = 0.096$)
Mixed Forest	26	*H. flava*	176	6.77	210	8.08	($P = 0.355$)	4.04	($P = 0.039$)
		H. longicornis	26	1.00	38	1.46	($P = 0.130$)	0.73	($P = 0.261$)
		I. nipponensis	19	0.73	27	1.04	($P = 0.465$)	0.52	($P = 0.402$)
Total	136	*H. flava*	422	3.10	704	5.18	($p < 0.001$)[e]	2.59	($P = 0.111$)
		H. longicornis	2,626	19.31	3521	25.89	($P = 0.200$)	12.94	($P = 0.140$)
		I. nipponensis	57	0.42	97	0.71	($P = 0.016$)[e]	0.36	($P = 0.429$)

[a] No. of paired replicates by dragging and sweeping for ticks.
[b] Mean no. of adults and nymphs collected by dragging vegetation/100 m² using a 1 m wide × 1.5 m long flannel cloth attached to a wooden pole behind the collectors as the collectors walked forward. Ticks were collected from the underside, then the topside of the flannel cloth at ≈5 m intervals. Approximate area sampled by each collector was 100 m (length × 1 m (width) = 100 m².
[c] Mean no. of adults and nymphs collected by sweeping vegetation/200 m² using a 1 m × 1 m flannel cloth attached to an aluminum pole that was swept from side to side as the collectors walked forward. Ticks were collected from the underside, then the topside of the flannel cloth at ≈5 m intervals. Approximate area sampled was estimated at 100 m (length) × 2 m (sweeping width) = 200 m².
[d] Mean no. of adults and nymphs collected over each transect by sweeping vegetation. (The area surveyed is based on a 2-m swath as the collector walked forward for 100 m distance with the mean no. ticks/100 m² = [mean no. of ticks collected/(2 m × 100 m)]/2).
[e] Mean no. of adults and nymphs collected by sweeping vegetation was significantly higher than sweeping vegetation over the same area ($P < 0.05$), but not significantly different that mean no. of ticks per 100 m² by paired *t*-test.

ber of nymphs observed during October followed high numbers of larvae collected during August (Fig. 2B,D). *I. nipponensis* was less frequently collected than either *H. longicornis* or *H. flava* (Fig. 2C). The highest mean numbers of adults and nymphs were collected during May–June, with few collected during the remaining months. Larvae were collected more frequently during July–September (Fig. 2D).

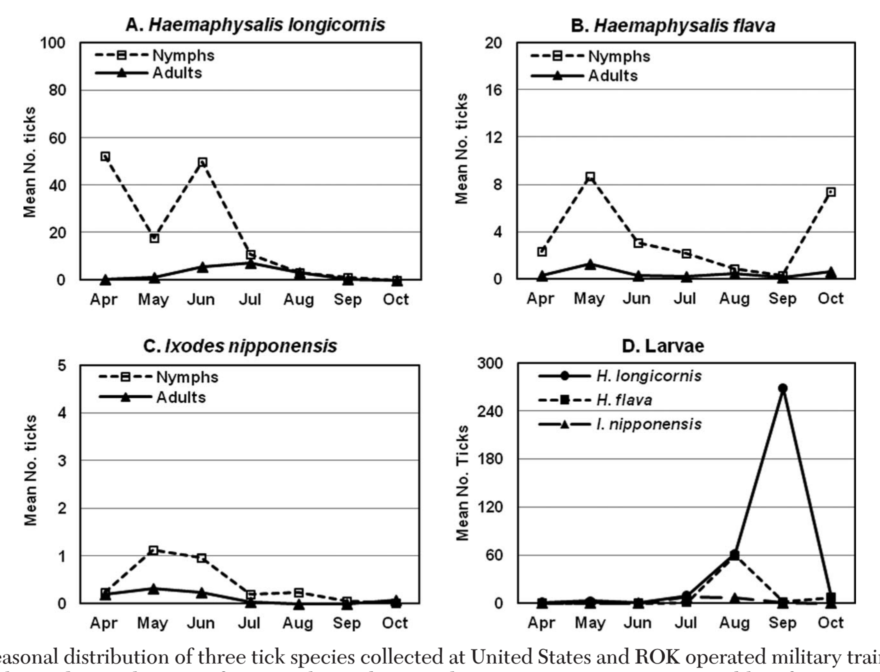

Fig. 2. Seasonal distribution of three tick species collected at United States and ROK operated military training sites and private lands located near the DMZ from April–October, northern Gyeonggi Province, Republic of Korea, 2004–2005.

Discussion

Tick-borne diseases pose serious health threats to U.S. military forces and civilian populations throughout the world, including the ROK (Merchant and Taboada 1991, Steen et al. 2004, Anna et al. 2012, Anonymous 2012). Over the past several years, there have been >5,000 cases of scrub typhus reported among Korean populations (Korean Centers for Diseases Control and Prevention [K-CDC] 2012), but few cases of tick-borne rickettsial infections, most likely because they are not reportable diseases and empirically (presence of an eschar) may be confused with scrub typhus. To support this, a recent retrospective serosurvey of ≈10,000 U.S. military personnel deployed to the ROK in the 1990s showed that 2.0% of the soldiers seroconverted to SFG *Rickettsia*, while only 0.2% seroconverted to scrub typhus (Richards 2009). These results were unexpected, because there have been no confirmed U.S. servicemen admitted to medical facilities in the ROK over the last several decades related to tick-borne pathogens. However, more recently in October 2012 and perhaps because of increased awareness, there was one confirmed (army soldier) and one empirical diagnosis (based on the presence of an eschar; military dependent) of scrub typhus reported, while there have yet to be confirmed cases of rickettsial disease. These results indicate disease risks associated with military training or recreational activities where there are unmanaged lands, including tall grasses and other herbaceous vegetation and cultivated/volunteer forests.

Since the Korean War (1950–1953), the ecology of the ROK has changed considerably from treeless hills and mountains that provided limited habitat and protection for medium to large mammals and forest dwelling bird populations. A national tree planting policy instituted in the 1960s resulted in a changed landscape, including military training areas, of planted groves of conifer and deciduous trees forested hillsides and mountains followed by forests of volunteer trees. The enrichment of flora provides for increased harborage, habitats, and diversity of small-large mammal populations (i.e., rodents, voles, squirrels, shrews, feral cats, dogs, raccoon dogs, deer, and wild pigs), and indigenous and migratory birds, in addition to the diversity and increased prevalence of associated ectoparasites and zoonotic pathogens.

Host associations are principal to the habitat distribution of ticks. *H. longicornis* was collected primarily in habitats consisting of grasses and other herbaceous vegetation and are associated with the large wild and domestic mammals that use these habitats. *H. flava* hosts range from small to medium-sized mammals and migratory and indigenous birds (Yamaguti et al. 1971; Kim et al. 2009a, 2010a). Hosts of *I. nipponensis* larvae and nymphs range from small to medium-sized mammals, while nymphs and adults blood feed on medium to large-sized mammals. Neither sweeping nor dragging methods were effective at collecting large numbers of *I. nipponensis* (unpublished data). However, for small mammal collections made in tall grasses and herbaceous vegetation in northern Gyeonggi Province, >98% of all ticks collected were *I. nipponensis* (3,771 larvae, 753 nymphs, 0 adults), while only one *H. flava* nymph was collected (Kim et al. 2010a). *I. nipponensis* adults and nymphs were more frequently collected in forest habitats, indicating their association with hosts that use forest habitats for refuge but perhaps feed or seek prey in open grass areas. Dragging and sweeping methods, while used extensively for the collection of ticks from vegetation, are much less useful in collecting nest species. In a separate report, *I. pomerantzevi* has been only reported from small mammals, while none were collected by dragging or sweeping even though attempts were made to collect them from areas where there were infested small mammals (Kim et al. 2010a).

The collection of ticks by various methods provides information that can be used for developing exposure and disease risk assessments based on population density estimates of questing ticks, risks of being bitten, and the prevalence and transmission of associated zoonotic pathogens. To accomplish this, a comprehensive tick-borne disease program that encompassed the collection of questing ticks from vegetation (sweeping, dragging, or CO_2 baited traps) and from small-large mammals and birds was established with the support of the Armed Forces Health Surveillance Center–Global Emerging Infections Surveillance and Response Systems (AFHSC–GEIS) and collaboration with local universities, ROK government agencies and AFHSC–GEIS partners. These data identify geospatial distribution of tick-borne disease threats and provide for the development of disease risk reduction strategies that impact on the medical readiness of U.S. personnel deployed to the ROK.

To evaluate the effectiveness of dragging and sweeping techniques, comparisons were made for the mean number of questing ticks collected to provide population abundance estimates, in addition to associated tick-borne pathogens (reported separately). Carroll and Schmidtmann (1992) showed that results for sampling nymph populations of the deer tick or blacklegged tick (*Ixodes dammini*, currently *I. scapularis*) in various habitats by sweeping and dragging techniques were similar. In simultaneous 100 m transects, sweeping was as effective as dragging for collecting *I. dammini* nymphs where understory vegetation was sparse. However, for habitats with dense vegetation, sweeping was twice as effective as dragging. Similarly, sweeping was significantly more effective for collecting *H. flava* and *I. nipponensis* nymphs and adults over a 100 m transect for deciduous and conifer forests, perhaps as a result of sweeping over scrub vegetation. However, when compared with the total area surveyed over the 100 m transect, there were no significant differences between dragging and sweeping. While sweeping may be more effective for collecting larger numbers of ticks under some conditions, it is physically more demanding, and especially in dense forest understory, movement is degraded and the aluminum poles occasionally break from stress. Therefore, dragging for ticks is the preferred method for efficiently collecting ticks from vegetation in the ROK.

Acknowledgments

We thank COL Hee-Choon Sam Lee, Chief, Force Health Protection and Preventive Medicine (FHP&PM), 65th Medical Brigade (MED BDE), for his dedicated support. We sincerely thank Suk-Hee Yi, FHP&PM, 65th MED BDE, for providing GIS maps of collection sites and data statistical analysis. We also thank Joel Gaydos, Armed Forces Health Surveillance Center–Global Emerging Infections Surveillance and Response System (AFHSC–GEIS), Silver Spring, MD, for his encouragement and support. Funding for this work was provided through the joint partnership between the AFHSC–GEIS, Silver Spring, MD, and the National Center for Medical Intelligence, Ft. Detrick, MD.

References Cited

Anonymous. 2012. Reported vectorborne and zoonotic diseases, U.S. Army and U.S. Navy, 2000–2011. MSMR 19: 15–16.

Alekseev, A. N., H. V. Dubinina, I. van De Pol, and L. M. Schouls. 2001. Identification of *Ehrlichia* species and *Borrelia burgdorferi* in *Ixodes* ticks in the Baltic Regions of Russia. J. Clin. Microbiol. 39: 2237–2242.

Anna, M. M., J. D. Escobar, and A. S. Chapman. 2012. Reported vectorborne and zoonotic diseases, U.S. Air Force, 2000–2011. MSMR 19: 11–14.

Carroll, J. F., and E. T. Schmidtmann. 1992. Tick sweep: modification of the tick drag-flag method for sampling nymphs of the deer tick (Acari: Ixodidae). J. Med. Entomol. 29: 352–355.

Chae, J. S., C. M. Kim, E. H. Kim, E. J. Hur, T. A. Klein, T. K. Kang, H. C. Lee, and J. W. Song. 2003. Molecular epidemiological study for tick-borne disease (*Ehrlichia* and *Anaplasma* spp.) surveillance at selected U.S. military training sites/installations in Korea. Ann. N.Y. Acad. Sci. 990: 118–125.

Chiba, N., M. Osada, K. Komoro, T. Mizutani, H. Kariwa, and I. Takashima. 1999. Protection against tick-borne encephalitis virus isolated in Japan by active and passive immunization. Vaccine 17: 1532–1539.

Daniels, T. J., T. M. Boccia, S. Varde, J. Marcus, J. Le, D. J. Bucher, R. C. Falco, and I. Schwartz. 1998. Geographic risk for Lyme disease and human granulocytic ehrlichiosis in southern New York State. Appl. Environ. Microbiol. 64: 4663–4669.

Falco, R.C. and D. Fish. 1992. A comparison of methods for sampling the deer tick, *Ixodes dammini,* in a Lyme disease endemic area. Exp. Appl. Acarol. 14: 165–173.

Fournier, P. E., H. Fujita, N. Takada, and D. Raoult. 2002. Genetic identification of rickettsiae isolated from ticks in Japan. J. Clin. Microbiol. 40: 2176–2181.

Ishikura, M., H. Fujita, S. Ando, K. Matsuura, and M. Watanabe. 2002. Phylogenetic analysis of spotted fever group rickettsiae isolated from ticks in Japan. Microbiol. Immunol. 46: 241–247.

Jang, W. J., J. H. Kim, Y. J. Choi, K. D. Jung, Y. G. Kim, S. H. Lee, M. S. Choi, I. S. Kim, D. H. Walker, and K. H. Park. 2004. First serologic evidence of human spotted fever group rickettsiosis in Korea. J. Clin. Microbiol. 42: 2310–2313.

Kee, S., K. J. Hwang, H. B. Oh, M. B. Kim, J. C. Shim, H. I. Ree, and K. S. Park. 1994. Isolation and identification of *Borrelia burgdorferi* in Korea. J. Korean Soc. Microbiol. 29: 301–310.

Kim, C. M., J. Y. Kim, Y. H. Yi, M. J. Lee, M. R. Cho, D. H. Shah, T. A. Klein, H. C. Kim, J. W. Song, S. T. Chong, et al. 2005. Detection of *Bartonella* species from ticks, mites and small mammals in Korea. Vet. Sci. 6: 327–334.

Kim, C. M., Y. H. Yi, D. H. Yu, M. J. Lee, M. R. Cho, A. R. Desai, S. Shringi, T. A. Klein, H. C. Kim, J. W. Song, et al. 2006. Tick-borne rickettsial pathogens in ticks and small mammals in Korea. Appl. Environ. Microbiol. 72: 5766–5776.

Kim, S. Y., S. M. Yun, M. G. Han, I. Y. Lee, N. Y. Lee, Y. E. Jeong, B. C. Lee, and Y. R. Ju. 2008. Isolation of tick-borne encephalitis viruses from wild rodents, South Korea. Vector-Borne Zoon. Dis. 8: 7–13.

Kim, H. C., S. J. Ko, C. Y. Choi, H. Y. Nam, H. Y. Chae, S. T. Chong, T. A. Klein, W. J. Sames, R. G. Robbins, and J. S. Chae. 2009a. Migratory bird tick surveillance, including a new record of *Haemaphysalis ornithophila* Hoogstral and Kohls 1959 (Acari: Ixodidae) from Hong-do (Hong Island), Republic of Korea. Syst. Appl. Acarol. 14: 3–10.

Kim, S. Y., Y. E. Jeong, S. M. Yun, I. Y. Lee, M. G. Han, and Y. R. Ju. 2009b. Molecular evidence for tick-borne encephalitis virus in ticks in South Korea. Med. Vet. Entomol. 23: 15–20.

Kim, H. C., S. T. Chong, W. J. Sames, P. V. Nunn, S. P. Wolf, R. G. Robbins, and T. A. Klein. 2010a. Tick surveillance of small mammals captured in Gyeonggi Province, Republic of Korea, 2004–2008. Syst. Appl. Acarol. 15: 100–108.

Kim, H. C., I. Y. Lee, S. T. Chong, A. L. Richards, S. H. Gu, J. W. Song, J. S. Lee, and T.A. Klein. 2010b. Serosurveillance of scrub typhus in small mammals collected from military training sites near the DMZ, Northern Gyeonggi-do, Korea, and analysis of the relative abundance of chiggers from small mammals examined. Korean J. Parasitol. 48: 237–243.

Kim, H. C., T. A. Klein, H. J. Kang, S. H. Gu, S. S. Moon, L. J. Baek, S. T. Chong, M. L. O'Guinn, J. S. Lee, M. J. Turell, et al. 2011. Ecological surveillance of small mammals at Dagmar North training area, Gyeonggi Province, Republic of Korea, 2001–2005. J. Vec. Ecol. 36: 373–381.

Klein, T. A., H. J. Kang, S. H. Gu, S Moon, S. H. Shim, Y. M. Park, S. Y. Lee, H. C. Kim, S. T. Chong, M. O'Guinn, et al. 2012a. Hantaan virus surveillance targeting small mammals at Dagmar North training area, Gyeonggi Province, Republic of Korea, 2001–2005. J. Vect. Ecol. 36: 381–2011.

Klein, T. A., H. C. Kim, S. T. Chong, M. L. O'Guinn, J. S. Lee, M. J. Turell, W. J. Sames, S. H. Gu, H. J. Kang, S Moon, et al. 2012b. Hantaan virus surveillance in small mammals at firing points 10 and 60, Yeoncheon, Gyeonggi Province, Republic of Korea. Vector-Borne Zoon. Dis. 12: 674–682.

Ko, S. J., J. G. Kang , S. Y. Kim, T. A. Klein, H. C. Kim, S. T. Chong, W. J. Sames, S. M. Yun, Y. R. Ju, and J. S. Chae. 2010. Prevalence of tick-borne encephalitis virus in ticks from southern Korea. J. Vet. Sci. 11: 197–203.

Korea Centers for Diseases Control and Prevention (K-CDC). 2012. Provisional cases of reported notifiable diseases. Publ. Hlth. Wkly. Rep. 5: 845–851.

Lee, J. H., H. S. Park, K. D. Jung, W. J. Jang, S. E. Koh, S. S. Kang, I. Y. Lee, W. J. Lee, B. J. Kim, Y. H. Kook, et al. 2003. Identification of the spotted fever group rickettsiae detected from *Haemaphysalis longicornis* in Korea. Microbiol. Immunol. 47: 301–304.

Lee, S. O., D. K. Na, C. M. Kim, Y. H. Li, Y. H. Cho, J. H. Park, J. H. Lee. S.K. Eo, T. A. Klein, and J. S. Chae. 2005.

Identification and prevalence of *Ehrlichia chaffeensis* infection in *Haemaphysalis longicornis* ticks from Korea by PCR, sequencing and phylogenetic analysis based on 16SrRNA gene. Vet. Sci. 6: 151–155.

Mahara, F. 1997. Japanese spotted fever: report of 31 cases and review of the literature. Emerg. Infect. Dis. 3: 105–111.

Merchant, S. R., and J. Taboada. 1991. Dermatologic aspects of tick bites and tick-transmitted diseases. Vet. Clin. North Am. Small Anim. Pract. 21: 145–155.

Murphy, G. L., S. A. Ewing, L. C. Whitworth, J. C. Fox, and A. A. Kocan. 1998. A molecular and serologic survey of *Ehrlichia canis,* E. chaffeensis, and *E. ewingii* in dogs and ticks from Oklahoma. Vet. Parasitol. 79: 325–339.

O'Guinn, M. L., T. A. Klein, J. S. Lee, H. C. Kim, L. J. Baek, S. T. Chong, M. J. Turell, D. A. Burkett, A. Schuster, I. Y. Lee, S. H. Yi, W. J. Sames, K. J. Song, J.W. Song. 2008. Ecological surveillance of small mammals at Firing Points 10 and 60, Gyeonggi Province, Republic of Korea, 2001–2005. J. Vect. Ecol. 33: 370–384.

Park, H. S., J. H. Lee, E. J. Jeong, T. K. Park, T. Y. Kim, J. S. Chae, J. H. Park, T. A. Klein, W. J. Jang, K. H. Park, et al. 2005. Differentiation of Anaplasmataceae through partial groEL gene analysis. Microbiol. Immunol. 49: 655–662.

Park, K.H., S.H. Lee, W.J. Won, W.J. Jang, and W.H. Chang. 1992. Isolation of *Borrelia burgdorferi,* the causative agent of Lyme disease, from *Ixodes* ticks in Korea. J. Korean Soc. Microbiol. 27: 307–312 (in Korean).

Richards, A. L. 2009. Infections with typhus, spotted fever and scrub typhus group Rickettsiae among US military personnel deployed to the Republic of Korea. 19th Asia Pacific Military Medicine Conference (19th APMMC), 6–10 April, 2009, Seoul, Korea.

Robbins, R. G., and J. E. Keirans. 1992. Systematics and ecology of the subgenus Ixodiopsis (Acari: Ixodidae: Ixodes). Thomas Say Foundation Monograph XIV. Entomological Society of America, Lanham, MD.

Sames, W. J., T. A. Klein, H. C. Kim, S. T. Chong, I. Y. Lee, S. H. Gu, Y. M. Park, J. H. Jeong, and J. W. Song. 2009. Ecology of Hantaan virus at Twin Bridges training area, Gyeonggi Province, Republic of Korea, 2005–2007. J. Vect. Ecol. 34: 225–231.

Sames, W. J., T. A. Klein, H. C. Kim, S. H. Gu, H. J. Kang, S. H. Shim, S. J. Ha, S. T. Chong, I. Y. Lee, A. L. Richards, et al. 2010. Serological surveillance of scrub typhus, murine typhus, and leptospirosis in small mammals captured at Twin Bridges training area, Gyeonggi Province, Republic of Korea, 2005–2007. Mil. Med. 175: 48–54.

Scoles, G. A., M. Papero, L. Beati, and D. Fish. 2001. A relapsing fever group spirochete transmitted by *Ixodes scapularis* ticks. Vector-Borne Zoon. Dis. 1: 21–34.

Steen, C. J., P. A. Carbonaro, and R. A. Schwartz. 2004. Arthropods in dermatology. J. Am. Acad. Dermatol. 50: 819–42.

Takada, N., H. Fujita, Y. Yano, W. H. Huang, and C. Khamboonruang. 1993. Serosurveys of spotted fever and murine typhus in local residents of Taiwan and Thailand compared with Japan. SE Asian J. Trop. Med. Publ. Hlth. 24: 354–356.

Terassini, F. A., F. S. Barbieri, S. Albuquerque, M. P. Szabo, L. M. Camargo, and M. B. Labruma. 2010. Comparison of two methods for collecting free-living ticks in the Amazonian forest. Ticks Tick-borne Dis. 1: 194–196.

Yamaguti, N., V. J. Tipton, H. L. Keegan, and S. Toshioka. 1971. Ticks of Japan, Korea, and the Ryukyu Islands. Brigham Young Univ. Sci. Bull. 15: 1–226.

Received 17 February 2012; accepted 14 January 2013.