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Differences in tick infestation intensity by season, sex, age class, and body region of feral raccoons (*Procyon lotor*) in the Miura Peninsula, Japan

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Abstract. The raccoon (*Procyon lotor*) is an invasive species in Japan and has been identified as a susceptible host and reservoir of tick-borne diseases. In our study conducted on the Miura Peninsula, Kanagawa Prefecture, Japan, a total of 47 604 ticks were collected from 253 raccoon carcasses (92.3% of raccoons). The predominant tick species found was *Haemaphysalis flava*, constituting 97.4% of the collected ticks, followed by eight other species from three genera. Generalized linear mixed models with raccoon sex, age, season, and land use at the captured site as explanatory variables indicated that raccoon sex and seasonal variations were significant factors influencing tick infestations across all stages and sexes of *H. flava*. Raccoon age indicated an impact on adult and larval *H. flava*. Additionally, the land use was relevant only for the model of nymph, with urban areas being a significant factor. Our analysis suggests that male raccoons have a substantial role for increasing and spreading ticks. We also noted a significant concentration of ticks infesting ears and head of raccoons. These findings contribute to the development of efficient and quantitative evaluation methods for tick infestation towards disease prevention strategies in wildlife management.

Key words: *Haemaphysalis flava*, host–parasite relationship, on-host tick attribute, tick collecting method.

Abstract in Japanese (要旨). 三浦半島のアライグマ (*Procyon lotor*) の季節、性、年齢、体表部位によるマダニ寄生状況の違い。日本国内において外来種アライグマ (*Procyon lotor*) は、マダニ媒介感染症病原体の感受性宿主、またはレゼルポア (感染巣) であることが確認されている。神奈川県三浦半島で実施した調査では、253頭のアライグマの死骸 (アライグマの92.3%) から47 604頭のマダニが採集された。マダニは *Haemaphysalis flava* が97.4%と最も多く、次いで他の3属8種が採取された。アライグマの性、年齢、季節、捕獲場所の土地利用状況を説明変数とした一般化線形混合モデルにより、*H. flava* の全ステージおよび性別において、アライグマの性別と季節がマダニの咬着数に影響を及ぼす有意な要因であることが示された。また、アライグマの年齢は *H. flava* の成ダニと幼ダニに影響を与えることが示された。さらに、土地利用区分はメス成ダニに関連し、都市部が有意な要因であった。分析の結果、オスのアライグマは、マダニの増加と拡散に大きな役割を担っていることが明らかになった。また、アライグマの耳介と顔部にマダニが集中して咬着していることも明らかになった。これらの知見は、野生動物管理による感染症予防対策に向けて、マダニの咬着状況を効率的かつ定量的に評価する手法において有用な知見となることが期待される。

Tick-borne diseases (TBDs) pose a significant public health concern as they are vector-borne pathogens transmitted by ticks, many of which are also known to be zoonotic. Notably, diseases like Lyme disease (Murase et al. 2013), tularemia (Fujita 2004), rickettsiosis (Mahara

1984), severe fever with thrombocytopenia syndrome (SFTS) (Takahashi et al. 2014), and Heartland virus (Brault et al. 2018) are among the serious health problems associated with ticks. The incidence of rickettsiosis and SFTS has notably risen over the past decade in Japan

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(National Institute of Infectious Diseases, 2019, 2020).

In several countries, including industrialized nations like Japan, the United States, and European countries, the incidence of TBDs has been emerging and spreading (Dantas-Torres et al. 2012; Okabe et al. 2019; Rochlin and Toledo 2020). Understanding the interaction between humans and ticks is crucial to comprehend how TBD patients were exposed to ticks. Host-parasite relationships between wildlife and ticks vary both inter- and intra-specifically (Kollars and Ladine 1999; Doi et al. 2021b; Kwak et al. 2021). In particular, the intra-specific difference in tick infestation patterns among the host attribute, such as sex, age classes, seasons of investigation, and environment of their home-range located would become crucial to the tick distributions and survivals (Kollars and Ladine 1999; Egyed and Lang 2020). The understanding of the impact of host species attribute on tick distribution should lead us to understand the TBD risk area based on the abundance and distribution of wildlife with different attributes.

Previous studies conducted in natural conditions have reported that the abundance of ectoparasites including ticks increases depending on host aggregation patterns influenced by resource availability (Monello and Gompper 2010). The reproductive status of hosts may also influence tick infestation intensity. A previous study suggested that maternal anti-parasitic behavior decreased as the number of female offspring increased (Neuhaus 2003). Higher infestation intensity in male hosts compared to females has been attributed to sexual differences in home range and behaviors (Randolph 1975; Ostfeld et al. 1996). However, another study also reported that the effect of host sex towards tick infestation was different among the host species (Kollars and Ladine 1999). As reported in these studies, the sex, age class, and reproductive status affect tick infestation status, and these effects are necessary to be considered for each host species to evaluate host behaviors and dispersion of ticks.

Changes in wildlife distribution due to changes in landscape and anthropogenic activities can also strongly influence the tick-human interface. In recent years, wildlife including alien species such as raccoons (*Procyon lotor*) have exploited the urban environment in close proximity to human residential areas (Ikeda 2004; Gehrt et al. 2010). It is therefore necessary to understand the TBD risk caused by the urban wildlife. However, most studies have shown concerns on traffic accidents and agricultural damage caused by urban wildlife, and fewer studies have advocated caution on the zoonotic diseases

that might occur among humans and domestic animals (Bengis et al. 2004; Bradley and Altizer 2007; Dantas-Torres et al. 2012).

The raccoon is an introduced alien species in multiple countries including Japan (Lutz 1996; Agetsuma-Yanagihara 2004; Beltrán-Beck et al. 2012). They have been imported as pet animals and become feral in multiple regions of Japan since the 1960s (Agetsuma-Yanagihara 2004; Ikeda 2015). Doi et al. (2018, 2021b), Fujita (2009), and Sanjuán et al. (2022) have reported infestations of native tick species in feral raccoons. Also, other studies indicated that feral raccoons were suspected to be reservoirs of multiple types of pathogens causing TBDs (Berrada et al. 2006; Fujita 2009; Myśliwy et al. 2022; Tatemoto et al. 2022). Notably, feral raccoons have successfully adapted to urban and peri-urban environments (Lutz 1996; Gehrt et al. 2010; Rosatte et al. 2010), potentially serving as carriers of ticks with established host-parasite relationships and, in turn, contributing to the spillover of tick-borne pathogens to humans and domestic animals.

Therefore, understanding the relationship between raccoons and ticks in Japan is important for gathering the basic information necessary for efficient vector control and elucidating the precise transmission cycle of TBDs. The previous studies have reported raccoon infestation with ticks and the possibility that raccoons can be ecological boosters that may accelerate the reproduction and survival rate of vector ticks in human residential areas (Kollars and Ladine 1999; Doi et al. 2021b). Although raccoons are suspected to be the interface of ticks and tick-borne diseases in humans, the knowledge on the relationship between the phenology of tick infestation and the natural history of feral raccoons is still limited. The quantitative analysis would reveal a deep understanding of the biology and ecology of tick infestation. While many studies on tick infestation often used qualitative methods, a previous study surveyed intensity of tick infestation on migratory birds showed that birds foraging on the ground can transport a greater number of ticks (Zajac et al. 2022). Also, Doi et al. (2021b) differentiated tick infestation intensity in two introduced mammals, raccoon and masked palm civet (*Paguma larvata*), and indicated raccoon may have greater risk to carry ticks than masked palm civet. These studies emphasized the necessity of quantitative analysis on tick infestation to assess the potential risk of TBDs which were harbored by ticks and wildlife.

In order to facilitate quantitative analysis, it is neces-

sary to establish an efficient method of tick collection. Ticks are known to search the spot to infest for their bloodmeal after they attach to the host. The previous studies of roe deer revealed that infestation of *Ixodes ricinus* was the most intensively observed in the neck region (Kiffner et al. 2010). A study of rodents reported that ticks were attached to the host body via the toes and nose and they infested the auricles of rodent hosts (Egyed and Lang 2020). The study focusing on the feral mongoose in Okinawa, Japan indicated that head region of the mongoose (*Urva auro-punctata*) was the most intensively infested region (Ishibashi et al. 2009). If the hotspots of infestation sites are identified, it will lead to the efficient collection of ticks and the proposal of simple quantitative evaluation methods.

In this study, we observed the distribution of tick infestations on raccoon body surfaces and assessed variations in tick infestation intensity based on host raccoon sex and age, seasonality, captured locations, seasonality, and land use status. This information serves as foundational knowledge for developing future tick investigation methods, including new tick collection techniques, and for planning vector control and monitoring strategies.

Materials and methods

Animal samples

We received 274 raccoon carcasses from Miura Peninsula district in Kanagawa Prefecture, Japan (Fig. 1). The raccoons were captured under the Kanagawa Prefecture Raccoon Control Implementation Plan from January 2015–April 2021 (Kanagawa Prefecture 2016). The captured raccoons were euthanized with carbon dioxide gas according to the previously established protocol (American Veterinary Medical Association 2001). The animal study protocol was approved by the Bioethics Committee of Nippon Veterinary and Life Science University (protocol code: 2022 K-3; approval date: January 31, 2022). The raccoon carcasses were preserved at -20°C until necropsy was performed.

Tick collection

We collected the ticks infesting the carcasses of 274 raccoons captured between January 2015 and April 2021 (Table 1). Part of the tick samples collected were included in the reports by Doi et al. (2018) ($n = 115$) and Doi et al. (2021b) ($n = 82$). Seventy-seven raccoons captured from January to March 2015, July 2016 to



Fig. 1. Map of the study area. The raccoons were captured in Hayama Town and Yokosuka City, Kanagawa Prefecture, Japan.

Table 1. Number of raccoon carcasses used for this study

| | Female | Male | Total |
|---------------------------|-----------|-----------|-----------|
| Weaned (≥ 5 month) | 95 (87) | 109 (102) | 204 (189) |
| Pre-weaned (< 5 month) | 37 (33) | 33 (31) | 70 (64) |
| Total | 132 (120) | 142 (133) | 274 (253) |

The number in brackets indicates the number of raccoons infested with one or more ticks.

October 2018, and February 2020 to April 2021 were newly collected in this study.

Ticks that infested 28 weaned raccoon carcasses (age class was explained in the statistical analysis section) were collected from January–March 2017. Due to the enormously labor-intensive method, we limited the collection to seasons when the tick infestation was the greatest (Doi et al. 2018). Ticks were collected from seven different body regions (ears, face, neck, dorsum, ventrum, tail, and limb areas) (Fig. 2). The raccoon carcasses were examined with forceps and a cat-flea comb until any individuals of the tick could not be found. All the collected ticks were preserved in 70% ethanol for morphological identification.

Tick identification

The ticks collected from raccoons were identified, and their genera and species, life stages, and sex were recorded based on morphological features using a stereomicroscope, biological microscope, and identification keys (Yamaguti et al. 1971; Fujita and Takada 2007).

Statistical analysis

We compared tick infestation intensity between the sexes and age classes. The age classes were divided into two groups based on tooth eruption and the time of separation of the juveniles from their mother: pre-weaned (< 5 months) and weaned (≥ 5 months) (Stuewer 1943; Montgomery 1964; Zeveloff 2002). We provided gener-

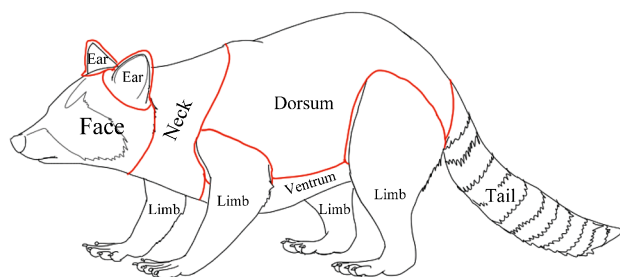


Fig. 2. Raccoon body regions. The definition of each body region is; Ear: auricles and approximately 2 cm in perimeter from the base of the auricle; Face: the anterior area from the neck circumference (the line connecting mandibular angle and transverse process of cervical vertebrae I), excluding the area of ear; Neck: the area between the neck circumference and the line connecting spinous process of C7, acromion, and clavicle; Dorsum: dorsal side of the body trunk between the lines connecting the axillary fossa and the groin and the area between the posterior end of neck and the base of tail; Ventrum: ventral side of the body trunk between the lines connecting the axillary fossa and the groin and the area between the posterior end of neck and the base of tail; Limbs: four appendages; Tail: from first coccygeal vertebrae to the tip.

alized linear mixed models (GLMMs) for each sex and developmental stage of *Haemaphysalis flava* ($n_{\text{adult male}} = 177$, $n_{\text{adult female}} = 170$, $n_{\text{nymph}} = 199$, $n_{\text{larva}} = 80$) (see Results). We set the number of *H. flava* as the response variable, utilizing a Poisson distribution with a log-link function. The explanatory variables included the sex and age classes of raccoons, as well as seasons. To account for seasons, we converted them into dummy variables based on the months when raccoons were captured: January to March as winter, April to June as spring, July to September as summer, and October to December as autumn, following the methodology outlined in the study of medium-sized wildlife in Miura Peninsula by Doi et al. (2021b) and Tei et al. (2011). Additionally, we incorporated land use information, considering the total area (m^2) of agricultural fields, forests, and urban areas in each tertiary geographical mesh of the raccoon capturing site (data provided by Kanagawa Prefecture), and calculated using the High-Resolution Land Use Land Cover Map Ver. 21.03, which comprises satellite images with a resolution of 10 m obtained from the Japan Aerospace Exploration Agency (2021). The center coordinates (latitude and longitude) of the tertiary geographical mesh (1 km^2) served as the location where raccoons were captured and were included as a random variable in the Generalized Linear Mixed Models (GLMMs). The significance of explanatory variables was assessed using *P*-values obtained through the Wald test for each variable, and we also conducted a Moran test to consider the

spatial autocorrelation.

The number of ticks attached to each body surface region of raccoons was compared using the Steel-Dwass test ($\alpha = 0.05$) for adult ticks and nymphal ticks among male and female raccoons. We scored +1 when the region was infested with a significantly greater number of *H. flava*. We scored -1 when the region was infested with a significantly smaller number of *H. flava*. We summed the score to compare among body regions. We did not analyze larval ticks because the number of larval ticks was very small during this season (Doi et al. 2018).

We used R (ver. 4.2.3) software with the R packages “glmmTMB”, “spatialreg”, and “spdep”, and function “Steel.Dwass” were used to perform statistical analyses (R Development Core Team 2023).

Results

Out of 274 raccoon carcasses, 47 604 individuals of three genera and eight species of ticks were collected from 253 raccoon carcasses (92.3%). *Haemaphysalis flava* was the most dominant species ($n = 46\,386$, 97.4%), followed by *Ixodes tanuki* ($n = 309$, 0.6%), *H. longicornis* ($n = 280$, 0.6%), *I. ovatus* ($n = 256$, 0.5%), *H. megaspinosa* ($n = 171$, 0.4%), *Amblyomma testudinarium* ($n = 10$, < 0.1%), *H. hystricis* ($n = 6$, < 0.1%), *H. japonica* ($n = 1$, < 0.1%), and *H. formosensis* ($n = 1$, < 0.1%). Due to damage to the specimens, 184 ticks were not identified (see Supplementary Table S1 for the number of ticks for each developmental stage). From the results, the number of species other than *H. flava* was excessively few. We therefore considered that conducting quantitative analysis would be unsuitable for species other than *H. flava*. Thus, we performed the following statistical analysis for *H. flava* only.

The dominant *H. flava* infestation in the raccoons was in the range of 0–3291 ticks per raccoon. The intense infestations of adult and nymphal *H. flava* were observed during winter and spring (Fig. 3A, B, and C). Larval *H. flava* was mostly observed during summer season (Fig. 3D).

The number of infesting adult male *H. flava* was significantly affected by sex and age class of raccoon, and season (winter and summer) (Table 2). The Moran test for adult male *H. flava* indicated insignificant spatial autocorrelation among the raccoon captured locations (Moran’s *I* statistic = -0.013, $P = 0.782$, Table 3). The number of infesting adult female *H. flava* was significantly affected by sex and age class of raccoon, season (winter, spring, and summer), and habitat use (urban)

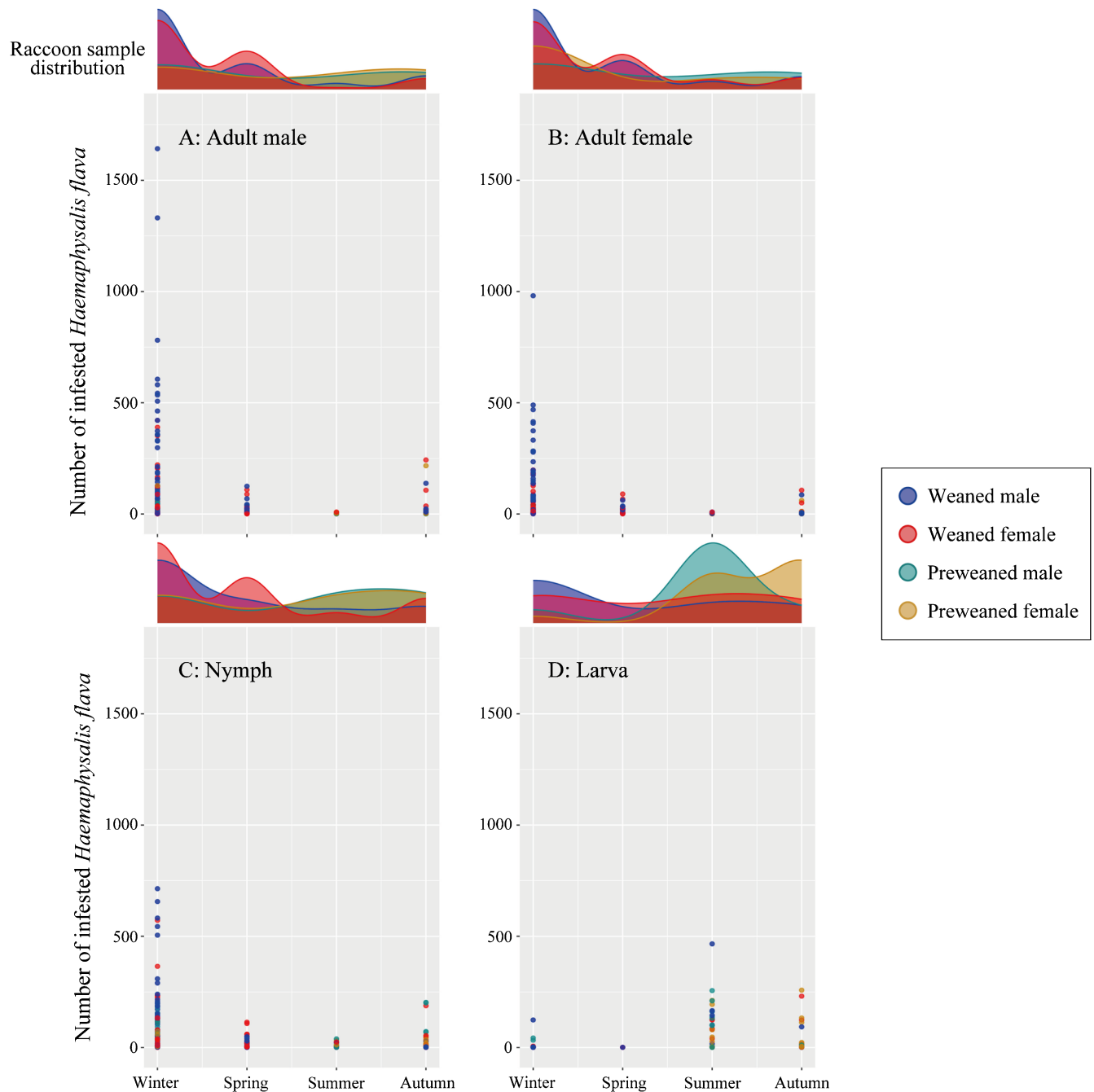


Fig. 3. The seasonal changes of *Haemaphysalis flava* (A: adult male, B: adult female, C: nymph, D: larva) infestation. Winter (January–March), Spring (April–June), Summer (July–September), and Autumn (October–December).

(Table 2). The Moran test for adult female *H. flava* indicated insignificant spatial autocorrelation among the raccoon captured locations (Moran's I statistic = -0.016 , $P = 0.809$, Table 3). The number of infesting nymphal *H. flava* was significantly affected by sex of raccoon and season (winter, spring, and summer) (Table 2). The Moran test for nymphal *H. flava* indicated insignificant spatial autocorrelation among the raccoon captured loca-

tions (Moran's I statistic = -0.010 , $P = 0.710$, Table 3). The number of infested larval *H. flava* was significantly affected by intercept, sex of raccoon, and season (winter, spring, and summer) (Table 2). The Moran test for larval *H. flava* indicated insignificant spatial autocorrelation among the raccoon captured locations (Moran's I statistic = -0.035 , $P = 0.713$, Table 3).

Comparison of tick infestation numbers among body

Table 2. Results of GLMMs for *Haemaphysalis flava* adult male, adult female, nymph, and larva

| | Explanatory variable | Estimate | SE | z value | 95% CI | | P value |
|---|---|-------------------------|------------------------|---------|-------------------------|-------------------------|-------------|
| | | | | | 2.5% | 97.5% | |
| Adult male/ <i>H. flava</i> (n = 177) | Intercept | 1.394 | 0.987 | 1.38 | -0.592 | 3.380 | 0.306 |
| | Sex (male = 1 / female = 0) | 0.995 | 0.020 | 49.35 | 0.956 | 1.035 | < 0.001 *** |
| | Age class (weaned = 1 / pre-weaned = 0) | 1.108 | 0.046 | 24.61 | 1.020 | 1.197 | < 0.001 *** |
| | Winter | 0.763 | 0.045 | 16.36 | 0.672 | 0.854 | < 0.001 *** |
| | Spring | -0.925 | 0.046 | -16.74 | -1.033 | -0.816 | 0.909 |
| | Summer | -2.924 | 0.114 | -13.03 | -3.363 | -2.484 | < 0.001 *** |
| | Forest | 1.529*10 ⁻⁶ | 1.031*10 ⁻⁶ | 1.44 | -5.476*10 ⁻⁷ | 3.606*10 ⁻⁶ | 0.149 |
| | Urban | -1.299*10 ⁻⁶ | 1.152*10 ⁻⁶ | -1.10 | -3.617*10 ⁻⁶ | 1.019*10 ⁻⁶ | 0.272 |
| | Agricultural land | -1.300*10 ⁻⁶ | 1.768*10 ⁻⁶ | -0.71 | -4.870*10 ⁻⁶ | 2.271*10 ⁻⁶ | 0.479 |
| | Adult female/ <i>H. flava</i> (n = 170) | Intercept | 1.793 | 1.101 | 1.630 | -0.365 | 3.952 |
| Sex (male = 1 / female = 0) | | 1.113 | 0.028 | 40.050 | 1.059 | 1.168 | < 0.001 *** |
| Age class (weaned = 1 / pre-weaned = 0) | | 1.384 | 0.068 | 20.250 | 1.250 | 1.517 | < 0.001 *** |
| Winter | | 1.116 | 0.063 | 17.640 | 0.992 | 1.240 | < 0.001 *** |
| Spring | | -0.416 | 0.074 | -5.620 | -0.562 | -0.271 | < 0.001 *** |
| Summer | | -1.750 | 0.161 | -10.890 | -2.065 | -1.435 | < 0.001 *** |
| Forest | | -1.517*10 ⁻⁷ | 1.126*10 ⁻⁶ | -0.130 | -2.359*10 ⁻⁶ | 2.056*10 ⁻⁶ | 0.893 |
| Urban | | -2.909*10 ⁻⁶ | 1.260*10 ⁻⁶ | -2.310 | -5.379*10 ⁻⁶ | -4.399*10 ⁻⁷ | 0.021 * |
| Agricultural land | | -2.849*10 ⁻⁶ | 1.764*10 ⁻⁶ | -1.620 | -6.307*10 ⁻⁶ | 6.078*10 ⁻⁷ | 0.106 |
| Nymph/ <i>H. flava</i> (n = 223) | | Intercept | 2.069 | 0.886 | 2.340 | -0.365 | 3.952 |
| | Sex (male = 1 / female = 0) | 0.656 | 0.020 | 33.340 | 1.059 | 1.168 | < 0.001 *** |
| | Age class (weaned = 1 / pre-weaned = 0) | 0.051 | 0.028 | 1.820 | 1.250 | 1.517 | 0.069 |
| | Winter | 0.767 | 0.043 | 17.990 | 0.992 | 1.240 | < 0.001 *** |
| | Spring | -0.718 | 0.053 | -13.610 | -0.562 | -0.271 | < 0.001 *** |
| | Summer | -2.020 | 0.081 | -24.880 | -2.065 | -1.435 | < 0.001 *** |
| | Forest | 1.570*10 ⁻⁷ | 9.157*10 ⁻⁷ | 1.710 | -2.359*10 ⁻⁶ | 2.056*10 ⁻⁶ | 0.087 |
| | Urban | -4.616*10 ⁻⁶ | 1.054*10 ⁻⁶ | -0.440 | -5.379*10 ⁻⁶ | -4.399*10 ⁻⁶ | 0.661 |
| | Agricultural land | -1.555*10 ⁻⁶ | 1.571*10 ⁻⁶ | -0.990 | -6.307*10 ⁻⁶ | 6.078E*10 ⁻⁷ | 0.322 |
| | Larva/ <i>H. flava</i> (n = 80) | Intercept | 4.332 | 2.485 | 1.743 | -0.540 | 9.203 |
| Sex (male = 1 / female = 0) | | 0.335 | 0.042 | 7.957 | 0.252 | 0.417 | < 0.001 *** |
| Age class (weaned = 1 / pre-weaned = 0) | | -0.369 | 0.037 | -9.998 | -0.441 | -0.296 | < 0.001 *** |
| Winter | | -3.458 | 0.245 | -14.144 | -3.937 | -2.979 | < 0.001 *** |
| Spring | | -3.835 | 0.730 | -5.251 | -5.266 | -2.403 | < 0.001 *** |
| Summer | | 0.837 | 0.067 | 12.557 | 0.706 | 0.968 | < 0.001 *** |
| Forest | | -7.526*10 ⁻⁷ | 2.529*10 ⁻⁶ | -0.298 | -5.709*10 ⁻⁶ | 4.203*10 ⁻⁶ | 0.766 |
| Urban | | -1.644*10 ⁻⁶ | 2.801*10 ⁻⁶ | -0.587 | -7.134*10 ⁻⁶ | 3.846*10 ⁻⁶ | 0.557 |
| Agricultural land | | -1.811*10 ⁻⁶ | 3.437*10 ⁻⁶ | -0.527 | -8.547*10 ⁻⁶ | 4.926*10 ⁻⁶ | 0.598 |

Asterisks: significantly different from 0 in Wald test (**P* < 0.05; ***P* < 0.01; ****P* < 0.001)

95% CI: 95% Confidence Interval

Std. Dev.: Standard Deviation

Table 3. Results of Moran test for GLMMs

| Stage/ Species | Moran <i>I</i> statistic standard deviate | <i>P</i> value | Moran's <i>I</i> statistic | Expectation | Variance |
|-------------------------------|---|----------------|----------------------------|-------------|----------|
| Adult male/ <i>H. flava</i> | -0.786 | 0.782 | -0.013 | -0.005 | 0.0001 |
| Adult female/ <i>H. flava</i> | -0.875 | 0.809 | -0.016 | -0.006 | 0.0001 |
| Nymph/ <i>H. flava</i> | -0.554 | 0.710 | -0.010 | -0.005 | 0.0001 |
| Larva/ <i>H. flava</i> | -0.561 | 0.713 | -0.035 | -0.018 | 0.0009 |

Table 4. The total number of *Haemaphysalis flava* infested each body region

| | | Ears | Face | Neck | Ventrum | Dorsum | Tail | Limbs |
|------------------------------------|---------------|------|------|------|---------|--------|------|-------|
| Male raccoon (<i>n</i> = 18) | Adult ticks | 1034 | 494 | 148 | 30 | 203 | 4 | 18 |
| | Nymphal ticks | 1295 | 424 | 52 | 51 | 59 | 2 | 21 |
| Female raccoon (<i>n</i> = 10) | Adult ticks | 219 | 70 | 27 | 18 | 93 | 1 | 21 |
| | Nymphal ticks | 299 | 167 | 28 | 82 | 22 | 5 | 18 |

regions indicated that the highest number of *H. flava* were observed on the ear region (*n* = 2847). This was followed by the face (*n* = 1155), dorsum (*n* = 377), neck (*n* = 255), ventrum (*n* = 181), limb (*n* = 78), and tail (*n* = 12) areas (Table 4). In the Steel-Dwass test, the male raccoon ear and face showed the highest score of the tick infestation among all the body regions in both sexes (Table 5, Supplementary Table S2). No significant difference in infestation numbers between the second-highest-scored female ear and the male ear was detected. However, the tick infestation in female ear tended to be less intense and did not indicate significant difference against the female ventrum. In other words, males tended to have tick infestation aggregated in ear and face, while females tended to have slightly less aggregated ticks (Table 5B, Fig. 4). Other species of ticks, *I. tanuki*, *H. megaspinoso*, and *H. longicornis* were collected from ear, face, and ventrum of male and female raccoons but the numbers of samples were not enough to apply for statistical analysis (Table 6).

Discussion

Tick collection from wildlife samples provides valuable insights into the parasite load in wildlife host species. In this study, we counted the number of ticks attached to individuals of raccoon. This information is crucial for understanding when and under what conditions certain raccoon individuals can disperse a higher number of ticks. This study detected differences in the *H. flava* infestation intensity according to the host attribute, sex, age class, seasonality, land-use of raccoons captured, and

body region of the raccoons.

Our GLMM analysis revealed significant effects of raccoon sex on the infestation intensities of all developmental stages of *H. flava* (Table 4). Specifically, male weaned raccoons exhibited higher infestations of male and female adult *H. flava*, while male pre-weaned raccoons had increased larval *H. flava* infestations. The contrasting patterns observed in the age class of raccoons in the GLMMs may be attributed to the seasonal dynamics of *H. flava* and raccoons as discussed below.

The notable surge in larval *H. flava* infestation during summer may be linked to reproductive seasonality (Kakuda et al. 1990). Our GLMM indicated that larval *H. flava* infestation was positively influenced by both season (summer) and age class of raccoons (male pre-weaned) and showed fewer infestation in spring and winter. This trend may be attributed to the behavior of male pre-weaned raccoons, which start roaming at 1–2 months, become more active at 3–4 months, and eventually leave maternal care at 5–6 months. Male raccoons also tend to cover longer distances compared to females (Zeloff 2002; Kamler and Gipson 2003; Gehrt et al. 2008; Kato et al. 2009; Rosatte et al. 2010; Hirsch et al. 2013; McWilliams and Wilson 2014). Raccoons in our study area are usually born in spring (Kato et al. 2009). The time when male raccoon cub starts roaming alone is the hatching season of larval *H. flava*. This increased mobility likely increases the chances of encounters between male pre-weaned raccoons and larval *H. flava*. Moreover, Doi et al. (2021b) discussed the difficulty of removing smaller-sized *H. flava* larvae, even with grooming, compared to engorged adult ticks. Male raccoon

Table 5. The score results of Steel-Dwass test for nymphal tick (A) and adult tick (B) infestation among body regions of male and female raccoon

A: Nymphal *H. flava*

| | F-ears | F-face | F-cervix | F-dorsum | F-ventrum | F-limbs | F-tail | M-ears | M-face | M-cervix | M-dorsum | M-ventrum | M-limbs | M-tail |
|-------------|--------|--------|----------|----------|-----------|---------|--------|--------|--------|----------|----------|-----------|---------|--------|
| F-ears | | 0 | -1 | -1 | 0 | -1 | -1 | 0 | 0 | -1 | -1 | -1 | -1 | -1 |
| F-face | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | -1 |
| F-cervix | 1 | 0 | | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| F-dorsum | 1 | 0 | 0 | | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| F-ventrum | 0 | 0 | 0 | 0 | | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| F-limbs | 1 | 0 | 0 | 0 | 0 | | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| F-tail | 1 | 0 | 0 | 0 | 0 | 0 | | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| M-ears | 0 | 0 | -1 | -1 | -1 | -1 | -1 | | 0 | -1 | -1 | -1 | -1 | -1 |
| M-face | 0 | 0 | 0 | 0 | 0 | -1 | -1 | 0 | | 0 | -1 | 0 | -1 | -1 |
| M-cervix | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | | 0 | 0 | 0 | 0 |
| M-dorsum | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | | 0 | 0 | 0 |
| M-ventrum | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | | 0 | 0 |
| M-limbs | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | | 0 |
| M-tail | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | |
| Total score | 9 | 1 | -2 | -2 | -1 | -3 | -3 | 10 | 5 | -2 | -3 | -2 | -3 | -4 |

B: Adult *H. flava*

| | F-ears | F-face | F-cervix | F-dorsum | F-ventrum | F-limbs | F-tail | M-ears | M-face | M-cervix | M-dorsum | M-ventrum | M-limbs | M-tail |
|-------------|--------|--------|----------|----------|-----------|---------|--------|--------|--------|----------|----------|-----------|---------|--------|
| F-ears | | 0 | 0 | 0 | -1 | -1 | -1 | 0 | 0 | 0 | 0 | 0 | -1 | -1 |
| F-face | 0 | | 0 | 0 | 0 | 0 | -1 | 1 | 0 | 0 | 0 | 0 | 0 | -1 |
| F-cervix | 0 | 0 | | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| F-dorsum | 0 | 0 | 0 | | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| F-ventrum | 1 | 0 | 0 | 0 | | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| F-limbs | 1 | 0 | 0 | 0 | 0 | | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| F-tail | 1 | 1 | 0 | 0 | 0 | 0 | | 1 | 1 | 1 | 1 | 1 | 0 | 0 |
| M-ears | 0 | -1 | -1 | -1 | -1 | -1 | -1 | | 0 | -1 | -1 | -1 | -1 | -1 |
| M-face | 0 | 0 | -1 | 0 | -1 | -1 | -1 | 0 | | 0 | 0 | -1 | -1 | -1 |
| M-cervix | 0 | 0 | 0 | 0 | 0 | 0 | -1 | 1 | 0 | | 0 | 0 | 0 | -1 |
| M-dorsum | 0 | 0 | 0 | 0 | 0 | 0 | -1 | 1 | 0 | 0 | | 0 | 0 | 0 |
| M-ventrum | 0 | 0 | 0 | 0 | 0 | 0 | -1 | 1 | 1 | 0 | 0 | | 0 | 0 |
| M-limbs | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | | 0 |
| M-tail | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | |
| Total score | 5 | 1 | -2 | -1 | -3 | -3 | -7 | 11 | 7 | 1 | 0 | -1 | -3 | -5 |

M-: Male raccoon, F-: Female raccoon.

Each body region was scored +1 when the region was infested with significantly greater number of *H. flava*, and scored -1 when the region was infested with significantly smaller number of *H. flava*. Score 0 indicated no statistically significant difference between the body regions.

cubs, spending more time roaming alone, may have fewer opportunities for grooming due to reduced maternal care, further contributing to their susceptibility.

Adult male *H. flava* showed significantly higher infestation in winter but not in spring. Conversely, adult

female *H. flava* exhibited significantly higher infestation during both winter and summer. This may reflect the reproductive strategy of *H. flava*, with increased winter populations and only females infesting raccoons until spring. This is likely due to mating during the overwin-

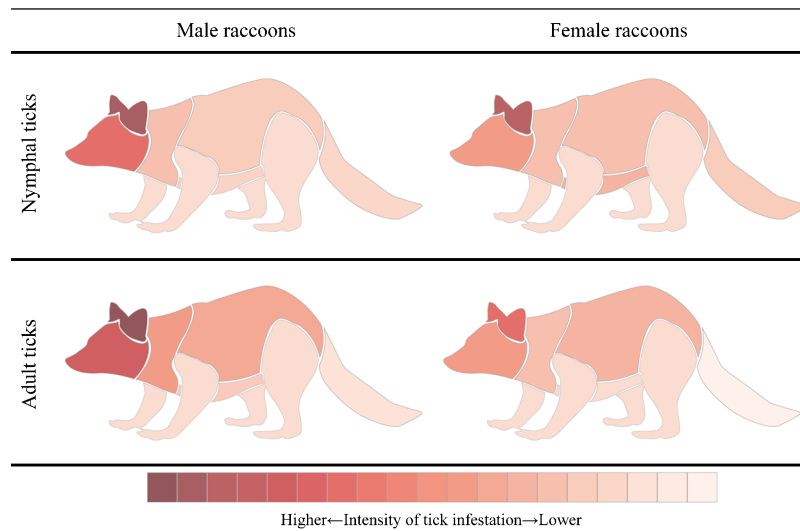


Fig. 4. The tick infestation distribution among male and female raccoons. The tick infestation intensity indicated in accordance with the results of Steel-Dwass test (see Tables 4 and 5).

Table 6. Infested body regions, raccoon sex, and number of collected tick species other than *Haemaphysalis flava*

| Stage/ Species | Body region | Raccoon sex | Number of collected ticks |
|-------------------------------------|-------------|-------------|---------------------------|
| Nymph/ <i>H. longicornis</i> | Ear | M | 1 |
| | | F | 1 |
| | Ventrum | M | 3 |
| Adult male/ <i>H. megaspinosa</i> | Ear | M | 1 |
| | Ventrum | M | 1 |
| Adult female/ <i>H. megaspinosa</i> | Face | M | 1 |
| Adult male/ <i>I. tanuki</i> | Ear | M | 1 |
| | | F | 1 |
| | Face | M | 2 |
| | | F | 1 |
| | Ventrum | M | 2 |
| Adult female/ <i>I. tanuki</i> | Ear | M | 1 |
| F | | 1 | |
| Face | | M | 4 |
| | | F | 4 |
| Ventrum | | M | 8 |
| Nymph/ <i>I. tanuki</i> | Ear | M | 1 |

tering period, after which males discontinue infestation, while females continue to infest raccoons for bloodmeals necessary for egg formation. The observed seasonal behaviors were consistent with those of *Dermacentor variabilis*, whose copulation and egg formation occur in

preparation for the incoming spring season, which is more suitable environment for eggs and hatching larvae to survive (Sonenshine and Stout 1971; Sonenshine 1979; Newhouse 1983; Carroll and Nichols 1986; Monello and Gompper 2007).

Meanwhile, infestation intensity of nymphal *H. flava* was not affected by age class of raccoon. Nevertheless, Kakuda et al. (1990) reported phenological patterns of nymphal and adult *H. flava* were similar, suggesting that a host preference might change between nymphal and adult stages. Guglielmone et al. (2014) noted that adult *H. flava* indicated less diversity of host spectrum. Thus, nymphal *H. flava* may have been attracted to other host species and was not affected by age class of raccoon significantly.

On another note, the land use variables showed no significant impact on the infestation intensity, except in the case of female *H. flava*, where the GLMM revealed that the habitat use (urban area) significantly affected the infestation. Since raccoons exhibit diverse habitat preferences, raccoons captured in forested areas may have spent an equal amount of time in urban or agricultural environments. Nevertheless, the GLMM for the female *H. flava* indicated that raccoons in urban environment were infested with fewer female *H. flava*. Yano et al. (1988) mentioned that eggs of *H. longicornis* need the relative humidity higher than 75.5% to hatch. Sonenshine and Tigner (1969) also noted necessity of humid environment for egg hatching of *A. americanum* and *D. variabilis*. Female *H. flava* may infest the host which

more frequently uses forest environment where the humidity is higher and more stable than the urban environment to increase egg hatching rate. Further research is needed to explore the relationship between location of hosts and infestation intensity for deeper understanding of ecology of ticks. Despite the GLMM results suggesting that urban raccoons carry relatively few female ticks, it is important to note that raccoons are known as carriers of various pathogens (Berrada et al. 2006; Kiuno et al. 2022; Tatemoto et al. 2022). Thus, the risk of infectious diseases in urban raccoons should not be underestimated.

Our results and previous studies indicated that the male raccoons could disperse a greater number of *H. flava* in larger areas than the female raccoons. Shaw et al. (2003) reported that tick-host encounter rates were influenced by home range size and activity levels. A previous study noted that the home range of male raccoon was between 1.66–3.35 km², whereas that of the female was 0.17 km² (Bartoszewicz et al. 2008). The male raccoons exert a greater influence on *H. flava* distribution than the female raccoons. Thus, the male adult raccoons in Miura Peninsula have stronger role as the ecological booster for *H. flava*, which provide blood-meals for adult male and female *H. flava* and contribute to increased reproduction of *H. flava* (Table 3) (Doi et al. 2021b).

While Moran tests among all of our GLMMs did not indicate significant spatial autocorrelation (Table 3). However, the tick fauna is often associated with climatic factor and interspecific wildlife host variations (Doi et al. 2021a). The larger scale comparison which can consider the differences of climatic factors and host fauna may indicate spatial effects.

Tick infestation data from wildlife are crucial for understanding vector tick distribution patterns. However, collecting ticks from captured wildlife can be time-consuming, leading some researchers to avoid it or report non-quantitative data. Our study offers a more efficient method to provide data on the distribution of tick infestation intensity on body surface of raccoons. We observed that *H. flava* primarily infested the head region of raccoons, including the ears and face (Fig. 2). Monello and Gompfer (2007) reported a similar pattern of *D. variabilis* infestation on head region of raccoons in the United States. In Okinawa, Japan, mongooses were also infested with endemic ticks, with intensive infestations observed on the neck and head regions (Ishibashi et al. 2009). Egyed and Lang (2020) reported that tick infestations in *Apodemus* species were often aggregated on auricles,

speculating that grooming behaviors might cause differences in tick infestation intensities among body regions. Other studies on roe-deer (*Capreolus capreolus*) suggested higher tick infestation intensity in the head and neck regions (Kiffner et al. 2010). However, research on *Rhipicephalus caudatus* infestation in cattle reported high tick infestation intensity in the dewlap, rump, fold of flanks, axillary, and inguinal regions (Kitaoka and Yamaji 1958). Aggregation of tick infestations may be influenced by host behavior, anatomy and characteristics of tick species. The infestation pattern among the body regions may be a result of grooming of host, so that ticks are remained and aggregated in the place where host cannot reach or ticks themselves may select a specific body region for their bloodmeal, or both. Collecting ticks from specific body regions is therefore useful for intraspecific comparisons of ticks in single species of host animal in the same geographical area. Additional cases are needed to test whether the collection of ticks from the face or ear regions is a viable method for obtaining quantitative tick infestation data in future studies.

Supplementary data

Supplementary data are available at *Mammal Study* online. **Supplementary Table S1.** Number of ticks collected from raccoon.

Supplementary Table S2. Results of Steel Dwass test on adult ticks.

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