

Effects of individual characteristics and seasonality and their interaction on ectoparasite load of Daurian ground squirrels in Inner Mongolia, China

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ABSTRACT

Understanding the drivers of parasite distribution is vital for ecosystem health, disease management, and vector monitoring. While studies note the impact of host sex, size, behavior, and season on parasite load, concurrent assessments of these factors and their interactions are limited. During the spring, summer and autumn seasons from 2021 to 2023, we trapped Daurian ground squirrel (*Spermophilus dauricus*), a small rodent species that inhabits eastern Asian grasslands in Inner Mongolia and collected their ectoparasites. Using machine learning Lasso regression, we pinpointed factors affecting tick and flea abundance on *S. dauricus*. We then analyzed these factors and their seasonal interactions with a mixed negative binomial generalized linear model. Our study revealed significant but inconsistent seasonal effects on the load of ectoparasites. The tick load was significantly higher in spring and summer compared to autumn, while the flea load was higher in summer and autumn but lacked statistical significance. Furthermore, individual factors that influence the flea and tick load were moderated by seasonal effects, with a male bias in flea parasitism observed in spring. Significant interactions were also found among seasonality, sex, and body weight. The load of male squirrel fleas was positively correlated with body weight, with the highest increase observed in spring. On the contrary, the flea load of female squirrels showed a negative correlation with body weight, significantly decreasing in the autumn with increasing weight. Significant interactions were observed between season and survival status, with hosts exhibiting higher tick load during autumn survival. Our findings underscore the importance of considering seasonal variation in parasitism and the interactions between seasonal dynamics and host biological traits in shaping parasite distributions.

1. Introduction

Rodents are responsible for crop damage, which diminishes agricultural yield, and they pose a significant risk to public health through the transmission of numerous zoonoses, such as plague, Hantavirus Pulmonary Syndrome, and Hemorrhagic Fever with Renal Syndrome (Liu et al., 2023a; Wu et al., 2021). Ectoparasites, including fleas and ticks, serve as vectors for pathogenic agents and are crucial in the transmission of diseases associated with rodents. Notably, plague, a significant global infectious disease caused by *Yersinia pestis*, is predominantly spread to humans by rodent-infesting fleas. This disease has

precipitated three pandemics, resulting in over 200 million fatalities. Recently, the potential for plague outbreaks has intensified due to climatic shifts and anthropogenic influences (Gao et al., 2021; Li et al., 2021; Liu et al., 2023a). In addition, parasites often play an important role in shaping host behavior, adaptation, and population dynamics (Hudson et al., 1998), and may even have a key influence on community structure in some ecosystems (Hatcher et al., 2014). Nevertheless, the distribution of parasites typically exhibits a non-random character. Therefore, investigating the spatial patterns of parasites and the underlying mechanisms is instrumental in enhancing our understanding of ecosystem processes and in informing strategies for disease mitigation,

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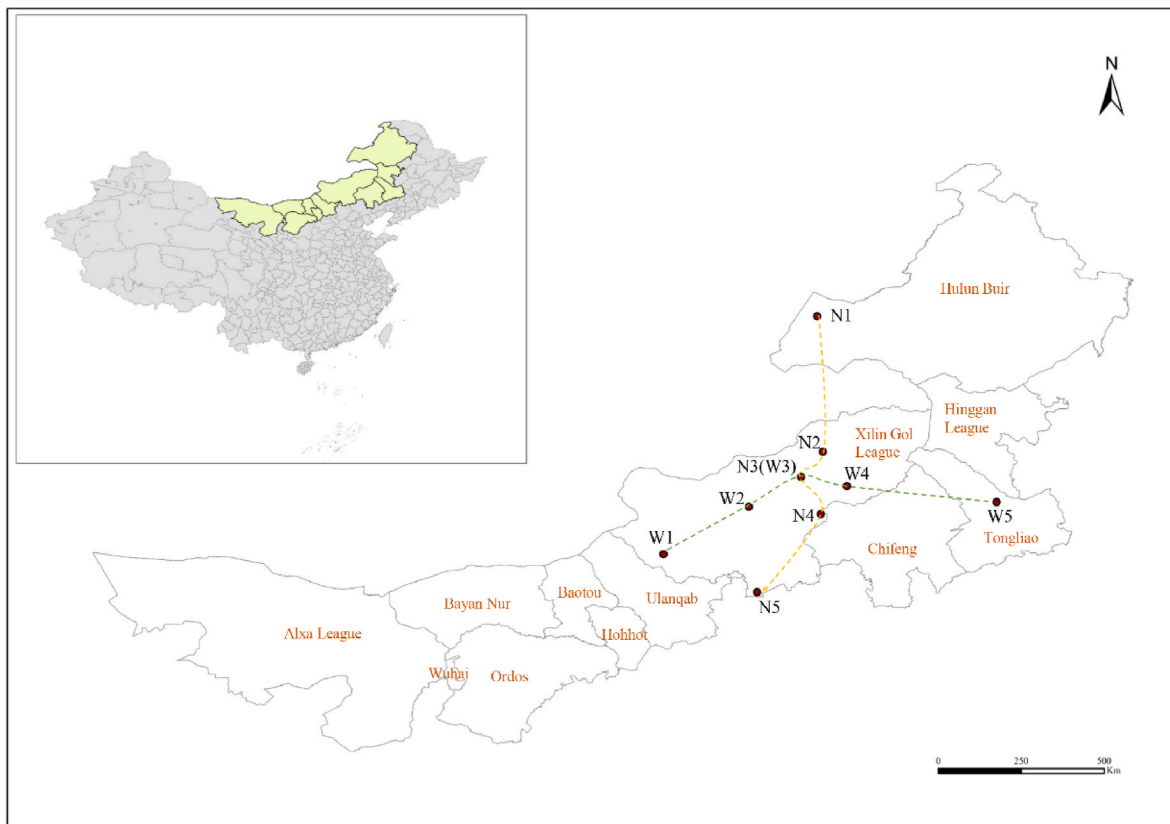


Fig. 1. Sample locations in Inner Mongolia. The sample sites are located in New Barag Right Banner (N1), East Ujimqin Banner (N2), Xilin Hot (N3/W3), Baiyin Siler (N4), Taipusi Banner (N5), Taibus Banner (W1), Sonid Right Banner (W1), Abag Banner (W2), West Ujimqin Banner (W4), and Tongliao (W5).

enhancing human health, advancing livestock management, and improving wildlife conservation efforts (Craig, 2003; Ma et al., 2023; Perkins et al., 2003; Reperant, 2010).

In the field of parasitology, an almost universal paradigm is the propensity of parasites to distribute in aggregated patterns (Poulin, 2007). This indicates that there are substantial differences among host individuals in terms of their exposure frequency or vulnerability to parasites, leading to considerable variation in the parasite burden across the host population (Wang et al., 2024a). A number of biological traits of hosts, including body size (Butler et al., 2020; Chu et al., 2019), personality (Boyer et al., 2010), and sex (Krasnov et al., 2005), have been identified as correlates of parasite load. For example, a commonly observed pattern is sex bias in parasite infestation. Most studies support that males are often more parasitized than females (de Mendonça et al., 2020; Herrero-Cófreces et al., 2021), this difference was possibly driven by host body mass or immunosuppression related to hormone levels (Zduniak et al., 2023). Furthermore, the probability of parasite load increased with body weight, and increased more with weight for males than for females. Larger individuals are more likely to provide a larger surface area or simply can withstand a higher number of parasites (Butler et al., 2020; Lindsø et al., 2023). Finally, reproductive and immunosuppressed periods may also contribute to parasite infection. It suggests that the increase in susceptibility is due to a decrease in endurance (Horn and Luong, 2021). However, few studies have comprehensively assessed the influence of individual factors such as morphology, sex, reproductive status, and survival status on susceptibility to host ectoparasite infection.

In addition to the host-related factors mentioned above, parasite-related factors and seasonal patterns also influence the pattern of parasite distribution (Krasnov and Matthee, 2010; Krasnov et al., 2005). Parasitism has evolved independently across taxa, leading to significant differences among parasites in morphology, life history, and strategies

for exploiting hosts. Fleas exhibit specific or opportunistic host preferences, while ticks possess a broad host range and can feed on various hosts (Krasnov et al., 2004; Obiegala et al., 2021). Even within the same host species, different parasites may exhibit varying distribution patterns and underlying mechanisms. Moreover, ectoparasite infection followed a seasonal variation, with a high prevalence of ticks in summer and a high prevalence of fleas in April–September (Smith et al., 2005). Therefore, comparing different parasitic patterns can help to understand and prevent vector-borne pathogens.

In this study, we investigated the impact of individual heterogeneity in Daurian ground squirrels (*Spermophilus dauricus*) on the burden of ectoparasitic fleas and ticks. *S. dauricus* is a medium-sized, typically seasonally breeding mammal, mainly found in the grasslands and deserts grasslands of Northeast China and Inner Mongolia (Ren et al., 2022; Yang et al., 2021). As a well-known host of fleas and ticks, *S. dauricus* often acts as an important transmitter of plague (Shuai et al., 2022; Tian, 2018). In an earlier investigation, we observed unique distributional trends for fleas and ticks on *S. dauricus*, indicating that the mechanisms governing parasitism might vary between these two categories of external parasites (Shuai et al., 2022). Therefore, using 3 years of field data from 9 sites: (1) What is the prevalence and species diversity of ectoparasitic fleas and ticks? (2) Are there seasonal variations in the biological correlates of parasite load? (3) Do ticks and fleas exhibit differences in abundance patterns and associated biological correlates?

2. Materials and methods

2.1. Ethics statement

Ethical approval for this study was obtained from the Ethics Committee of the National Institute for Control and Prevention of Communicable Diseases, Chinese Center for Disease Control and Prevention.

Table 1
Sampling information of different populations of striped hamster.

Pop	Sites	Longitude	Latitude	Elevation(m)	Samples
N1	New Barag Right Banner, Hulun Buir	116.85 °E	48.81 °N	552	7
N2	East Ujimqin Banner, Xilin Gol	116.59 °E	45.60 °N	916	0
N3(W3)	Qog UI, Xilin Gol	115.92 °E	44.18 °N	947	134
N4	Baiyin Siler, Xilin Gol	116.78 °E	44.08 °N	1215	8
N5	Taibus Banne, Xilin Gol	115.17 °E	41.71 °N	1468	186
W1	Sonid Right Banner, Xilin Gol	112.64 °E	42.74 °N	1104	30
W2	Abag Banner, Xilin Gol	114.97 °E	44.03 °N	1144	111
W4	West Ujimqin Banner, Xilin Gol	116.73 °E	44.59 °N	968	3
W5	Horqin Left Middle Banner, Tongliao	121.55 °E	44.27 °N	203	24

Rodents were captured in designated areas for rodent control, which were neither privately owned nor protected. No specific permits were required for the described field studies.

2.2. Study area

The study was carried out on the grasslands of Inner Mongolia Autonomous Region, China. The region has relatively high terrain, with an average elevation of around 1000 m. The climate is characterized by a temperate continental monsoon climate, with abundant sunshine, concentrated but uneven precipitation, strong winds, and dramatic temperature fluctuations. Inner Mongolia stretches 2400 km from east to west, with a precipitation gradient of 300 mm per year. It also has the largest north-south distance of 1700 km, with an annual temperature difference exceeding 10 °C. To ensure effective intersite comparisons, we considered both the east-west direction (precipitation gradient) and the north-south direction (temperature gradient) when selecting optimal sampling locations. We identified 9 sampling points (Fig. 1, Table 1). Temperature and precipitation data were obtained from Worldclim at a resolution of 5 km² (Liu et al., 2023b).

2.3. Rodent and ectoparasite screening

During a three-year period (2021–2023), we conducted nine sampling events, each occurring three times a year in May (spring), July (summer) and September (autumn). For each sampling event, we placed 300 mousetraps baited with fresh peanuts at 5-m intervals within an area of approximately 0.05 square kilometers. In addition, we selected a fixed area with a high density of rodent population for each site, which varied in size from 1 to 1.5 ha. We deployed 100 Sherman live traps, each equipped with fresh peanuts as bait. These traps were distributed across the study area, with a focus on positioning them near burrow entrances. To ensure adequate sample sizes for each population, traps remained in place for one to two days per location. The operational period was set from 05:00 to 20:30 (Beijing time), aligning with the most active hours of *S. dauricus*. Traps were checked every 2 h. During each inspection, we replaced the occupied traps with new ones and refilled the bait as needed. The captured *S. dauricus* was immediately placed in a separate cotton bag to prevent parasites from escaping and then transferred to our laboratory for subsequent examinations. The impact of dead *S. dauricus* on parasite counts among individuals was relatively consistent and thus had no significant effect on the final results.

The cloth bags containing the rodents were placed in covered plastic containers. Cotton balls soaked in isoflurane anesthesia solution were added to the containers and sealed. The rodents were anesthetized for at least 20 min to ensure that both the rodents and their ectoparasites on the body surface were anesthetized and deceased (Leung et al., 2021; Yin et al., 2023). After anesthesia, the rodents were removed from the cloth bags and placed on a white porcelain plate. Using tweezers and a fine comb, we carefully scanned the body surface and the bag to check for ticks or fleas on each Daurian ground squirrel. Ticks and fleas collected from rodent bodies were immediately preserved in separate centrifuge tubes containing 95% ethanol. After collecting ectoparasites,

we measured the body length, tail length, ear height, and hind foot length using a ruler. The sex was determined based on the distance between the external genitalia and the anus. We recorded the weight, sex, and reproductive status of each Daurian ground squirrel. The rodents were dissected and we weighed body weight, spleen weight, epididymis and testes weight, and uterus and ovary weight. The collected ectoparasites were identified at the species level based on their morphological characteristics under a microscope (Liu and Shi 2009). Fleas in Inner Mongolia. Inner Mongolia People's Publishing House, Hohhot). Following the guidelines provided by Emel'yanova and Hoogstraal (1973), we documented the life stage of each tick (larva, nymph, or adult) (Emel'yanova and Hoogstraal, 1973).

2.4. Statistical analyzes

To identify the most crucial individual variable that influences the parasite load, we utilized the least absolute shrinkage and selection operator (LASSO) regression model (Laufer et al., 2023; Ohanyan et al., 2022). LASSO regression is a variable screening method known for its robust model stability. It achieves this by applying a penalty term during model estimation, which continuously compresses coefficients (Wang et al., 2024b). This approach simplifies the model effectively while addressing issues such as overfitting and multicollinearity. This approach shrinks the coefficients of the variables with minimal impact on the overall model to zero, thereby enhancing the predictive performance. Cross-validation was used to evaluate the model and select parameters, dividing the data set into 10 subsets for iterative training and testing. This iterative process assesses model performance and identifies optimal parameter values. During cross-validation, plotting a curve against lambda values allows visualization of model performance across different settings. The lambda value corresponding to "minimum deviation" denotes the optimal bias that best fits the data. Additionally, a risk prediction nomogram model was constructed using key parasite load-related variables, validated for its discriminatory power in forecasting stroke risk through receiver operating characteristic (ROC) curve analysis. Packages 'glmnet' and 'stabsel' in R were used to fit the LASSO model and for stability selection respectively (Ohanyan et al., 2022).

For the variables screened by lasso regression, we used the negative binomial generalized linear mixed effects model (GLMM) to fit flea load and tick load respectively (Kumle et al., 2021). Fixed terms of the flea model included the main effects of sex, season, body weight (centralized), body length, tail length, and the two-way interactions between season and other factors. The fixed terms of the tick model included the main effects of sex, season, survival, body length, as well as the two-way interactions between season and other factors. The site (nested within season) was included as a random term. We used the glmer.nb function from the R package 'lme4' to construct a negative binomial GLMM. To assess multicollinearity, we utilized the 'vif' function from the 'car' package. For terms with degrees of freedom greater than 1, we computed generalized variance inflation factors (GVIF), adjusted for the dimension of the confidence ellipsoid; for terms with 1 degree of freedom, variance inflation factors (VIF) were calculated (Cheng et al., 2022). Terms with VIF (or GVIF) values greater than 10, indicating high

Table 2
The species and quantity distribution of parasitological fleas and ticks in vitro of the *Spermophilus dauricus* during 2021–2023.

	species	quantity	Prevalence(%)	
Flea				
2021(1149)	<i>Citellophilus tesquorum</i>	929	63.00	
	<i>Neopsylla pleskei</i>	83	19.00	
	<i>Frontopsylla luculenta</i>	125	28.00	
	<i>Ophthalmopsylla kukuschkini</i>	5	2.50	
	<i>Neopsylla bidentatiformis</i>	4	1.50	
	<i>Nosopsyllus laeviceps</i>	3	1.00	
	2022(759)	<i>Citellophilus tesquorum</i>	600	64.46
		<i>Neopsylla pleskei</i>	46	13.86
		<i>Frontopsylla luculenta</i>	99	30.72
		<i>Ophthalmopsylla kukuschkini</i>	1	0.60
<i>Neopsylla bidentatiformis</i>		11	2.41	
<i>Leptopsylla pavlovskii</i>		1	0.60	
2023(512)	<i>Xenopsylla conformis conformis</i>	1	0.60	
	<i>Citellophilus tesquorum</i>	372	52.55	
	<i>Neopsylla pleskei</i>	47	8.76	
	<i>Frontopsylla luculenta</i>	50	13.87	
	<i>Neopsylla bidentatiformis</i>	41	8.03	
2023(512)	<i>Nosopsyllus laeviceps</i>	2	0.73	
	Tick			
2021(1213)	<i>Haemaphysalis verticalis</i>	560	54.00	
	<i>Dermacentor nuttalli</i>	653	56.50	
2022(1374)	<i>Haemaphysalis verticalis</i>	634	54.22	
	<i>Dermacentor nuttalli</i>	740	56.02	
2023(1660)	<i>Haemaphysalis verticalis</i>	746	54.74	
	<i>Dermacentor nuttalli</i>	914	59.12	

multicollinearity, were sequentially removed. This process continued until all remaining model terms had VIF (or GVIF) values below 10. Consequently, we excluded the interaction terms of ‘season * body length’ from the flea load model and the tick load model to mitigate multicollinearity. Model selection relied on the corrected Akaike Information Criterion (AICc) using the ‘dredge’ function from the ‘MuMIn’ package. Given that no single best model (with delta AICc greater than 2.00) was evident, we performed conditional model averaging with the ‘model.avg’ function from ‘MuMIn’ (Luke, 2017). All statistical analyses were performed in R version 4.2.3 and a two-tailed P-value <0.05 was considered statistically significant.

3. Result

A total of 503 *S. dauricus* (185, 267 and 51 individuals caught in spring, summer and autumn, respectively) were captured, with a total of 4247 ticks (spring: mean 4.04 ± s.d.8.48 ticks per *S. dauricus*; summer: 14.54 ± 25.50 ticks per *S. dauricus*; autumn: 2.13 ± 5.86 ticks per *S. dauricus*) and 2420 fleas (spring: mean 4.36 ± s.d. 10.63 fleas per *S. dauricus*; summer: 6.50 ± 10.85 fleas per *S. dauricus*; autumn: 9.76 ± 11.20 fleas per *S. dauricus*) collected. The diversity of ectoparasitic ticks was low, only *Haemaphysalis verticalis* and *Dermacentor nuttalli*. We identified 7 flea genera and 8 species. *Citellophilus tesquorum* accounted for 78.55% of fleas, *Frontopsylla luculenta* for 11.32%, and *Neopsylla pleskei* for 7.27%. Other species contributed less than 5% of the individuals. The abundance ranking was similar over the years. Among these ground squirrels, 62.82% and 68.99% of the individuals were infested with ticks and fleas, respectively (Table 2).

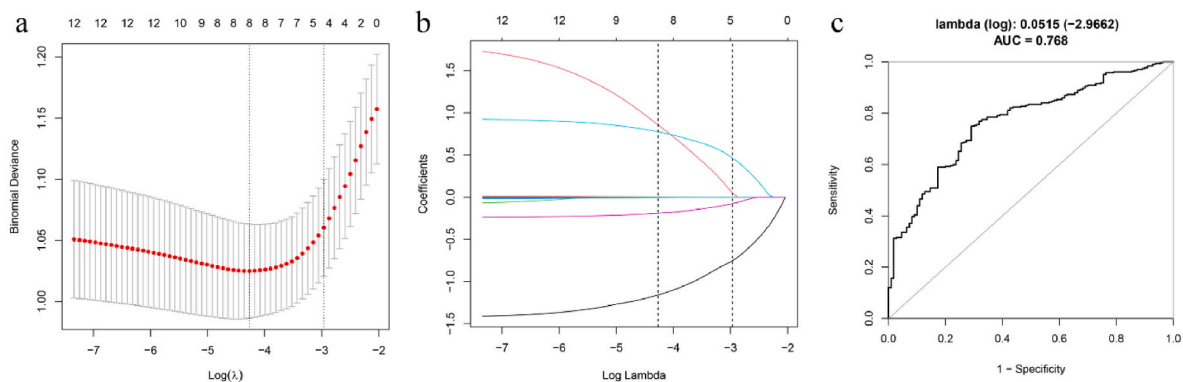


Fig. 2. The variables affecting flea load were screened based on Lasso regression. (a) The variation characteristics of the coefficient of variables, five variables were selected when $\log(\lambda) = -2.9662$; (b) the selection process of the optimum value of the parameter λ in the Lasso regression model by 10-fold cross-validation method; (c) Roc curve corresponding to $\log(\lambda)$.

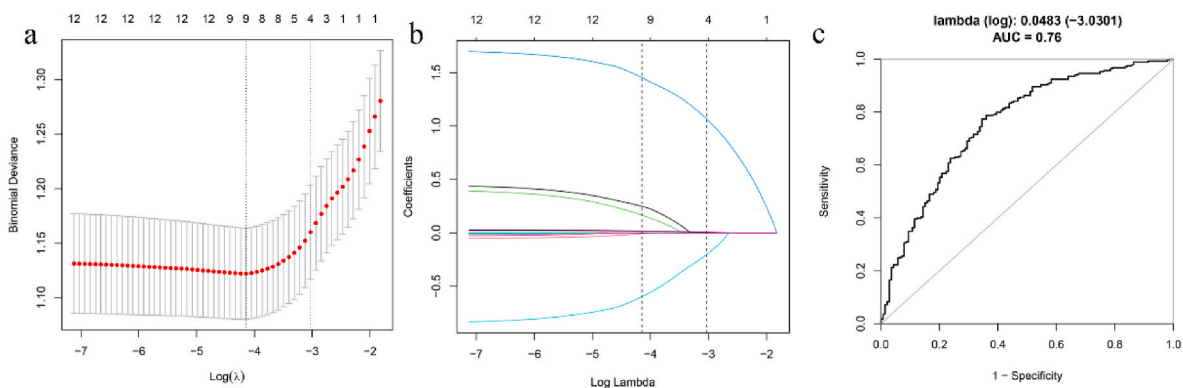


Fig. 3. The variables affecting tick load were screened based on Lasso regression. (a) The variation characteristics of the coefficient of variables, four variables were selected when $\log(\lambda) = -3.0301$; (b) the selection process of the optimum value of the parameter λ in the Lasso regression model by 10-fold cross-validation method; (c) Roc curve corresponding to $\log(\lambda)$.

Table 3

Results of conditional model averaging, based on negative binomial generalized linear mixed-effects model on ectoparasite load (whole data set) of Daurian ground squirrels (*Spermophilus dauricus*). Values of body weight were centralized prior to regressions. Terms in brackets show reference categories for categorical factors.

Dependent variable	Factor	Estimate	Standard error	Z value	P value	
Flea load (theta = 0.65)	Intercept	4.400	1.468	2.997	0.003	
	Sex	-1.363	0.497	-2743	0.006	
	Season (summer)	-1.247	1.185	-1.053	0.292	
	Season (autumn)	-2.651	2.313	-1.146	0.252	
	Weight	-0.017	0.007	-2.301	0.021	
	Length	-0.003	0.005	-0.671	0.503	
	Tail	0.027	0.024	1.119	0.263	
	Season:sex	0.552	0.266	2.075	0.038	
	Season: weight	0.008	0.004	2.046	0.041	
	Season:tail	-0.015	0.012	-1.259	0.208	
	Tick load (theta = 0.47)	Intercept	4.471	1.188	3.764	<0.001
		Sex	0.385	0.542	0.710	0.477
Season (summer)		0.290	0.725	0.499	0.617	
Season (autumn)		-2.533	1.213	-2.089	0.036	
Survive		2.476	0.686	3.608	<0.001	
Length		-0.007	0.005	-1.413	0.158	
Season:sex		-0.413	0.294	-1.405	0.160	
Season: survive		1.259	0.378	3.330	0.001	

We incorporated 12 factors that could potentially influence the parasite load, including season, survival status, sex, body weight, spleen weight, testes-epididymis weight, uterine-ovary weight, body length, ear height, hind foot length, tail length, and location, into a LASSO logistic model. Through a 10-fold cross-validation, we selected the optimal regularization parameter (λ). As depicted in Fig. 2a, the x-axis above represents the number of selected variables, while the y-axis indicates the model error rate. The lower x-axis denotes the values of λ for different harmonics, with λ_{\min} (left) and λ_{1se} (right) delineated by dashed lines, indicating the range where the model is most parsimonious. The values of the area under the curve (AUC) of the model fluctuate with changes in the logarithm of the harmonic parameter $\log(\lambda)$. Utilizing the LASSO logistic regression model, we built a risk factor classifier (Fig. 2b) and performed a ROC curve analysis (Fig. 2c). The maximum AUC value occurred at $\log(\lambda) = -2.9662$ ($\lambda = 0.0515$) for the flea load, leading to the selection of five influencing factors: season, sex, body weight, body length, and tail (Table S2). Similarly, for tick burden, the optimal $\log(\lambda)$ was found to be -3.0301 ($\lambda = 0.0483$), identifying four influencing factors: season, sex, survival status, and body length (Fig. 3abc, Table S1).

According to the results of conditional model average (Table 3), the flea load showed a significant sexual difference, with the flea load in the males significantly higher than in the females (Fig. 4a). There was a weak negative correlation between body weight and flea load. Furthermore, we observed an interactive effect on flea load between season and sex (Fig. 4b). The flea load was higher in males than in females, but this was statistically significant only in spring. The season also interacted with body weight to affect the flea load. Therefore, we constructed a three-way interaction graph of season, sex, and body weight, and found that body weight was positively correlated with flea load in males, and flea load increased the most with an increase in weight in spring, while in females, body weight was negatively correlated with flea load, and flea load decreased the most with an increase in weight in autumn (Fig. 4c).

The tick load also shows significant seasonal differences, with a higher load observed in spring and summer compared to autumn

(Fig. 5a). We found an interaction between season and survival status; during autumn, the tick load in live individuals was higher than in deceased individuals after capture, while this difference was not statistically significant during spring and summer (Fig. 5bc).

4. Discussion

The distribution of flea and tick infestations on Daurian ground squirrels suggests that parasitism is influenced by a complex interplay of various factors rather than by any single significant host or environmental variable. This insight has implications for the methodology of parasitism research, as investigating the combined effects of host and environmental characteristics on parasitism will provide a clearer understanding of the underlying mechanisms behind aggregation trends. Our research has found: (1) Male-biased effect of flea parasitism occurs in the spring season; (2) Among males, the flea load is positively correlated with body weight, with the greatest increase observed in spring. In contrast, among females, the flea load negatively correlates with body weight, with the most significant decrease occurring in autumn as body weight increases. (3) Ectoparasitic ticks exhibit a seasonal effect, predominantly concentrated in spring and summer. With hosts exhibiting a higher tick load when alive in autumn. Parasitism was primarily explained by different combinations of variable host traits (sex, body weight, length and survival state). Stochastic, geographic and environmental effects (season) were also important in how those mediated other factors. These interactions will be further explored in the future experimental work to better reveal the mechanisms of ectoparasites aggregation.

Sex-biased infestation of parasites in most mammalian hosts has been repeatedly reported (de Mendonça et al., 2020; Herrero-Cófreces et al., 2021; Krasnov et al., 2005; Morales-Montor et al., 2004). In our study, we found that in vitro flea load of *S. dauricus* was higher in males than in females. The hypotheses of male-biased parasitism are explained mainly in the following two aspects. Firstly, heightened male mobility enhances the likelihood of encountering parasites and increases both intraspecific and interspecific host contacts, thereby facilitating parasite transmission. Secondly, variations in hormonal status can influence anti-parasitic defense mechanisms due to the immunosuppressive effects of androgens (Krasnov et al., 2012). It would result in higher fitness of parasites exploiting males rather than females. For example, experimental studies have shown that the Reproductive capacity of flea parasites, at least in *Meriones crassus*, is greater when they feed on male rodents compared to females (Khokhlova et al., 2009). Therefore, manifestation of sex differences in parasite infestation of the same host may be depend on a variety of parasite-related and host-related factors (Krasnov and Matthee, 2010).

Body size stands as a primary and readily apparent characteristic of animals. Consequently, the exploration of its connections with various physiological, ecological, and behavioral attributes has been a subject of broad scientific inquiry. These researches have illuminated numerous correlations between body size and the diverse features of species (Kiffner et al., 2013; Peters, 1983). However, comparative studies on body weight as a determinant of parasite infestation have produced inconsistent findings. A study using a large data set found that body weight could produce positive or negative effects on parasitism (Cassin Sackett, 2018). Recent studies have found that in voles and wood mice, the infestation of parasites and the probability infection increased with body weight, with a more pronounced effect observed in males compared to females (Lindsø et al., 2023). Our study observed a positive correlation between flea load and body weight among males, with the highest increase observed during spring. In contrast, among females, there was a negative correlation between flea load and body weight, with the most significant decrease observed in autumn as body weight increased. One of the mechanisms behind this could be larger male ground squirrels may incur higher rates of parasitic exposure due to their expansive home ranges, which could render them more susceptible

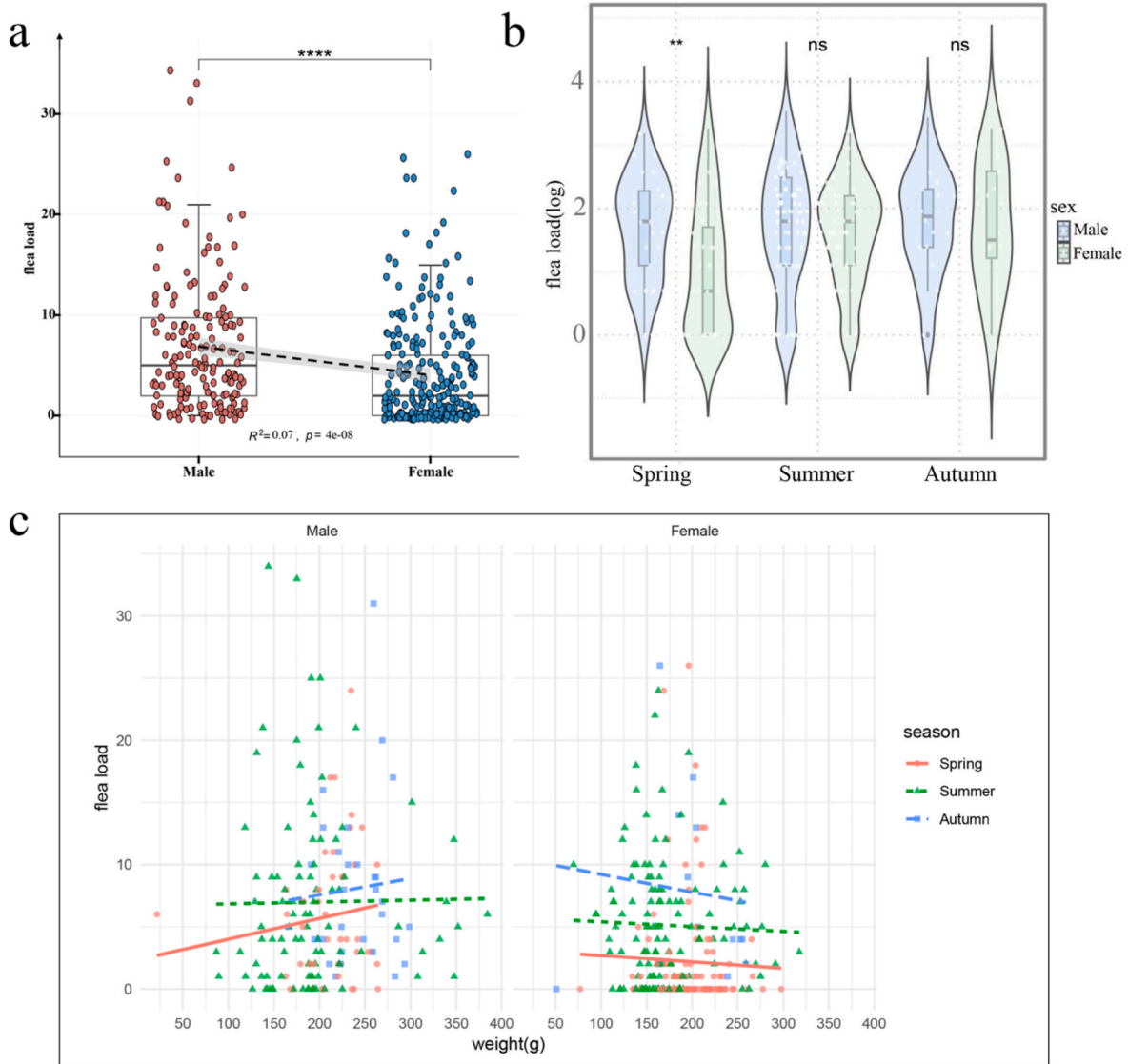


Fig. 4. (a) The differences of flea load between male and female *S. dauricus*; (b) Interactive effects of season and sex on flea load in *S. dauricus*; (c) Interactive effects of season and body weight across sex on flea load in *S. dauricus*.

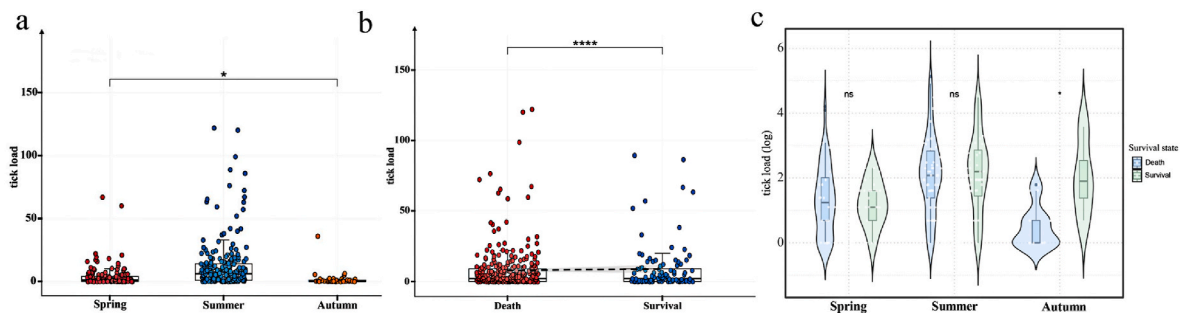


Fig. 5. (a) The difference of tick load in different seasons of *S. dauricus*; (b) The difference of tick load in different survival state of *S. dauricus*; (c) Interactive effects of season and survival state on tick load in *S. dauricus*.

to parasites, particularly during the spring when social and reproductive activities intensify (Shuai et al., 2022). Another reason is that increased body size in males may serve to accommodate a greater biomass of parasites by offering enhanced resources such as sustenance and habitat (Kiffner et al., 2013). In addition, high levels of androgens in males of high body weight, which may diminish their immune defenses against

fleas (Folstad and Karter, 1992), further substantiate the hypothesis that the effects of sex and body weight are anticipated to have an additive impact.

The extent, occurrence, and direction of sex-biased parasitism can vary not only between different hosts, parasites, or parasite-host associations, but also within the same parasite-host association across

different time periods. Our research has shown that the male-biased effect of flea parasitism in *S. dauricus* is particularly evident during the spring season, which may be associated with the seasonal activity patterns of certain flea species. Specifically, our study identified *Citellophilus tesquorum* as the predominant parasitic flea, peaking between March and May (Medvedev et al., 2019; Zhang et al., 2023). Furthermore, rodents exhibit varying spatial and burrowing behaviors across seasons, with heightened sex differences in territorial behavior observed during reproductive periods, typically occurring from late March to mid-May (Fan et al., 2022). Male rodents show increased mobility during breeding seasons, which correlates with androgen-driven parasitic effects. This finding aligns with previous research by Krasnov et al., which noted sex differences in flea parasitism patterns in rodent hosts within the Negev Desert, primarily during reproductive periods (Krasnov et al., 2004). During the breeding season, males exhibit tighter connectivity in their underground networks and possess larger home ranges, thereby engaging more frequently in indirect transmission and carriage of fleas. It is also plausible that females mitigate increased exposure through grooming behavior (Smith et al., 2018). In contrast, some studies have not detected significant sex differences in parasitism, potentially attributable to variations in host and parasite taxa as well as environmental factors (Abdel-Rahman et al., 2020; Baláz et al., 2023; Kiffner et al., 2013).

Habitat changes in most regions are seasonal and driven by fluctuations in temperature and precipitation. Additionally, host behaviors, physiology, and population dynamics also exhibit seasonal variations. Consequently, parasitic load shows temporal variations, typically characterized by seasonal fluctuations in ectoparasite loads (Le Coeur et al., 2015). In our study, significant seasonal differences in parasite load were identified, particularly evident in distinct patterns between fleas and ticks. Tick infestation rates were generally higher during spring and summer, whereas flea infestations peaked predominantly in summer and autumn. These seasonal patterns likely stem from inherent differences in the life histories and behaviors of fleas and ticks (Blagburn and Dryden, 2009). During the warm and humid summer and autumn seasons, conditions are ideal for flea reproduction. Furthermore, rodents are more active during this period, seeking food and building nests in preparation for hibernation, thus increasing the opportunities for flea infestation. *Dermacentor nuttalli* (*D. nuttalli*) (Wang et al., 2021) emerges as the predominant tick species affecting *S. dauricus* mongolicus. The seasonal dynamics of this tick species reveal adult prevalence in spring, with nymphs and larvae predominating during summer. Thus, seasonal variations in *Dermacentor* infestations in the prairies largely reflect reproductive patterns.

Different capture methods can lead to variations in host behavioral traits and survival status, potentially resulting in differences in parasite infestation loads. Therefore, it is expected that the surviving host individuals would exhibit higher parasite loads. In our study, we not only observed a higher tick load in surviving individuals at capture, but also observed significant interactions between tick load and host survival status across seasons. Specifically, only the survival status in the autumn was positively correlated with tick infestation, while no significant differences were found in other seasons. We attribute this finding to ticks preferring warm and humid environments, with Inner Mongolia experiencing lower temperatures in September, increasing ticks' temperature regulation needs (Viljoen et al., 2011). Moreover, sudden temperature drops after the deaths of Daurian ground squirrel likely led to increased energy constraints, reducing the external tick load and potentially influencing seasonal patterns of tick infestation (Smith et al., 2021). Additionally, variations in the number of Daurian ground squirrels captured using different methods (trap netting vs. cage netting) during our captures could introduce biases in the results. Currently, the literature on the influence of different capture methods on the ectoparasite load is limited. Although our study confirmed impacts on ticks, no flea effects were found. This difference may arise from distinct life histories and durations of attachment to the host between the two taxa. Fleas

typically remain associated with the host's nest, periodically attaching to the host for feeding and/or transmission. In contrast, ticks detach from the host and reappear in nearby vegetation after undergoing developmental stages, returning to the host for subsequent stages (Matthee and Krasnov, 2009). Further experiments are needed to validate these hypotheses.

In conclusion, our study emphasizes the role of seasonality in the formation of ectoparasite loads in rodents, as well as the seasonal variability of certain biological traits in hosts. Research also indicates significant differences in the distribution patterns and underlying mechanisms among different parasites, many of which carry zoonotic pathogens. Therefore, these findings provide information on vector competence for further investigation and guide rational allocation of resources in endemic regions to effectively control major parasites.

CRediT authorship contribution statement

Xiaoxu Wang: Writing – original draft, Visualization, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Meng Shang:** Writing – review & editing, Project administration. **Zihao Wang:** Writing – review & editing, Methodology. **Haoqiang Ji:** Supervision, Resources, Conceptualization. **Zhenxu Wang:** Writing – review & editing, Formal analysis, Data curation. **Guangju Mo:** Writing – review & editing, Project administration, Methodology. **Qiyong Liu:** Writing – review & editing, Visualization, Supervision, Funding acquisition, Conceptualization.

Data availability statement

Original contributions featured in the study are provided in the article/supplementary material. For additional inquiries, please contact the corresponding author.

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Declaration of interest statement

I declare that the authors have no competing interests as defined by BMC, or other interests that might be perceived to influence the results and/or discussion reported in this paper.

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Appendix A. Supplementary data

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