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Research Article

Spatio-seasonal variation in Hyalomma aegyptium infestation of Testudo graeca in the Sénalba Chergui forest of Djelfa (Algeria): main and interactive effects of body size, sex, and microhabitat

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Abstract:

This study aims to analyze the biological and environmental factors associated with infestation by the tick Hyalomma aegyptium in the tortoise Testudo graeca in the Sénalba Chergui forest (Djelfa, Algeria). A total of 150 tortoises were examined, and 1128 ticks were collected. Taxonomic identification demonstrated that all tick specimens belonged exclusively to the species Hyalomma aegyptium. The overall prevalence rate was determined to be 97.33%, while mean intensity and mean abundance were estimated at 7.73 and 7.52 ticks per individual, respectively. Infestation of Testudo graeca by Hyalomma aegyptium in the Sénalba Chergui forest is nearly generalized, intense, and strongly structured by ecological and biological conditions. Analysis of variance (ANOVA) revealed significant effects of season, sex, and their interaction on prevalence, mean intensity, and mean abundance. Analysis based on a generalized linear model with a Poisson distribution demonstrated that parasitic infestation increased considerably with increasing body size and varied according to microhabitat. The highest values were observed in microhabitats characterized by litter, shelters, and shrub cover. Furthermore, the chi-square test revealed a significant variation in parasite load according to study sites and seasons. However, the interaction between body size and microhabitat did not show an overall significant effect. The uniqueness of this study resides in its focus on interaction effects, especially between season and sex, underscoring that parasite dynamics are shaped not only by individual biological and environmental factors but also by their interactions. These results suggest that infestation dynamics are based on a close interaction between parasite phenology, host ecology, and environmental heterogeneity. By emphasizing the determining role of habitat microconditions in the maintenance and intensification of parasitism.

1. Introduction

Chelonians constitute one of the vertebrate groups most threatened worldwide due to habitat degradation, overexploitation, and the pet trade. (Stanford et al., 2020). The spur-thighed tortoise, *Testudo graeca*, represents a particularly relevant model for studying the interactions between conservation, population ecology, and parasitism. *Testudo graeca*, a long-lived species closely associated with generally vulnerable terrestrial ecosystems, faces constant anthropogenic pressure across many parts of its natural range, particularly through illegal capture and captivity for the pet trade. This highlights the importance of examining the factors likely to affect its biological condition and demographic dynamics (Segura et al., 2020).

Ticks are of particular importance because they can influence the physiological condition of their hosts, alter certain demographic parameters, and contribute to the spread of pathogens of veterinary concern, some of which may also be zoonotic (Segura et al., 2023), particularly in species like *Testudo graeca* that are already under stress from environmental pressures. In tortoises of the genus *Testudo*, *Hyalomma aegyptium* is recognized as the principal ectoparasite and the dominant tick species throughout much of the western Palearctic range. This parasite has a three-host life cycle: adults are closely associated with terrestrial tortoises of the genus *Testudo*, whereas larvae and nymphs are less host-specific and exploit a wider range of vertebrates, making it a highly relevant model for studies in parasite ecology and eco-epidemiology (Široký et al., 2006, 2011; Rubel, 2025).

Hyalomma aegyptium is of scientific interest both because of its high frequency on *Testudo graeca* and because of its potential role as a reservoir or vector of pathogens. Studies have shown its involvement in the persistence or transmission of certain microorganisms; experimental transmission has been well established for *Coxiella burnetii*, whereas for *Borrelia turcica*, available evidence mainly indicates transstadial transmission.

In Algeria, ticks collected from *Testudo graeca* have revealed the presence of an AP92-like variant of the Crimean-Congo hemorrhagic fever virus. In its natural habitat, *Hyalomma aegyptium* appears to be a highly specialized tick on adult tortoises and may be involved in more complex transmission cycles. These findings justify particular interest in studies conducted in North Africa (Široký et al., 2010; Kalmár et al., 2015; Kautman et al., 2016; Rubel, 2025).

Studies conducted to date in Morocco, Tunisia, the Balkans, and Algeria have shown that the infestation rate of *Testudo graeca* by *Hyalomma aegyptium* varies considerably depending on locality, habitat structure, host density, season, sex, age, and body size (Aouragh et al., 2020; Segura et al., 2019; Segura et al., 2023). However, information concerning the steppe and forest habitats of central Algeria remains incomplete. The combined effect of host biological factors and local ecological heterogeneity is still poorly understood. The aim of this study is to characterize the population of *Testudo graeca* in the Sénalba Chergui forest (Djelfa), identify the associated tick species, assess the key epidemiological parameters of infestation, and analyze the influence of factors such as season, sex, body size, microhabitat, and spatial variation on parasite burden. We hypothesize that infestation by *Hyalomma aegyptium* depends both on the individual characteristics of the host and on local ecological conditions, with higher infestation levels occurring during periods of greater seasonal activity.

2. Materials and Methods

2.1 Study area

The natural forest of Sénalba Chergui is located approximately 3 km northwest of the city of Djelfa, in the central region of the Ouled Naïl Mountains, within the Algerian Saharan Atlas. It extends approximately between 36°36′ and 36°42′ N and between 3°00′ and 3°12′ E, with a maximum altitude of about 1300 m. This forest belongs to the semi-arid bioclimatic zone, with an annual rainfall of 300 to 400 mm (Chakali, 2007), hot and dry summers, and cold winters. The vegetation is dominated by Aleppo pine (*Pinus halepensis*), accompanied by an understory composed mainly of *Quercus ilex*, *Juniperus phoenicea*, and *Juniperus oxycedrus*. Due to its transitional position between steppe forest and open habitats, Sénalba Chergui represents a site of major ecological interest for the study of biodiversity, vegetation, and host–parasite interactions in the semi-arid environments of the Djelfa region.

2.2 Sampling

The study was carried out at four sampling stations distributed within the Sénalba Chergui forest of Djelfa (Algeria), selected because of their environmental heterogeneity. Station 1 is located in a dense forest area, favorable for maintaining higher soil moisture and the presence of abundant litter. Station 2 corresponds to a semi-open area, more exposed to solar radiation and characterized by intermediate vegetation cover conditions. Station 3 is in a shrubland and bushy area, likely to promote tortoise concentration due to the availability of shelters. Finally, Station 4 is characterized by a rocky and dry environment, less favorable to moisture retention and the persistence of protected microhabitats. This sampling design was chosen in order to compare the levels of parasitic infestation observed in Testudo graeca under contrasting ecological conditions within the Djelfa forest.

2.3 Tortoise capture method and tick collection

Specimens were captured manually. The population size of *Testudo graeca* was estimated using the capture-mark-recapture (CMR) method, which is widely used in reptile ecology to assess the abundance and density of natural populations. This approach consists of capturing an initial sample of individuals, marking them individually, and then releasing them at the point of capture before conducting a second survey to record the number of marked individuals recaptured (Stubbs et al., 1984). This method is especially good for land tortoises because it gives a more accurate estimate of population size than just counting them by sight, especially when the animals are hidden or partially hidden by plants. Population size can then be calculated using the Lincoln-Petersen estimator as modified by Chapman, which corrects for bias associated with small sample sizes (Chapman, 1951). Sampling was carried out in 2024 during the active seasons of *Testudo graeca*, namely spring, summer, and autumn, while the winter season was excluded because of the species' low activity. In each of the four selected stations, four surveys were conducted per season following a standardized sampling effort. Sex determination of tortoises was based on external morphological characteristics, particularly plastron shape, tail length, and cloacal position. For each captured tortoise, the maximum anterior and posterior widths of the carapace and its maximum height, as well as the maximum straight anterior and posterior widths, were measured. Total body mass was measured using an electronic balance with a precision of 0.1 g, and all other dimensions were taken using a caliper.

Tick collection from terrestrial tortoises was carried out through a systematic macroscopic examination of each captured individual. All body parts likely to harbor ticks were carefully inspected, particularly the limbs, neck, tail, plastron, and skin junction areas, which are known to be preferred attachment sites. Detected ticks were removed manually using fine forceps and then preserved in tubes containing 70% ethanol. The samples were subsequently examined in the laboratory to determine the total number of ticks, their sex, developmental stage, and species, using the appropriate morphological keys (Apanaskevich, 2003; Meddour-Bouderda and Meddour, 2006; Hosseini-Chegeni et al., 2013). This procedure made it possible to estimate the main parasitological indicators, namely prevalence (%) = number of infested tortoises × 100 / total number of tortoises), mean intensity (number of ticks / number of infested tortoises), and mean abundance (number of ticks / total number of tortoises) (Bush et al., 1997; Segura et al., 2023).

2.4 Statistical analysis

The parasitological parameters of infestation by *Hyalomma aegyptium* in *Testudo graeca*, namely prevalence, mean intensity, and mean abundance, were calculated for the entire sample. The effects of season, host sex, and their interaction on these three variables were then assessed using factorial analyses of variance (ANOVA). To examine the effect of body size and microhabitat on parasite burden, a generalized linear model (GLM) with a Poisson distribution was fitted, also including the interaction term between these two factors. In addition, the combined effect of sampling station and season on the distribution of parasite burden was tested using a chi-square test of independence, complemented by the calculation of Cramer's V in order to estimate the strength of the association. Results were considered statistically significant at $p < 0.05$. These statistical analyses were performed using XLSTAT.

3. Results

3.1 Description of the tortoise population studied in the Sénalba Chergui forest of Djelfa

During the three monitoring seasons, a total of 150 individuals of the species *Testudo graeca* were recorded and examined across four sampling stations in the Sénalba Chergui forest (Djelfa). Surveys revealed a generally heterogeneous spatial distribution of the population across the study area. The variations in relative abundance observed among stations appear to be closely correlated with local ecological characteristics, particularly the structure of the vegetation layer, substrate properties, microclimate, and access to trophic and water resources.Analysis of the demographic structure revealed a predominance of adult individuals (98%), contrasting with the very low proportion of juveniles (2%). These observations indicate that the distribution and structuring of this population are strongly determined by habitat quality and the degree of habitat conservation.

3.2 Tick species composition in *Testudo graeca* in the Sénalba Chergui forest of Djelfa

The parasitological study conducted on a sample of 150 tortoises recorded a total of 1,128 ticks. Taxonomic analysis revealed that this ectoparasitic infestation was exclusively due to *Hyalomma aegyptium*, which was the only species identified among the collected specimens. The demographic analysis of these ectoparasites showed a marked predominance of adult individuals. Nymphs were observed only sporadically, whereas larvae were completely absent. This tick population displayed a pronounced sex imbalance, with a sex ratio ranging from 6 to 8 males per female.

3.3 Assessment of infestation factors by *Hyalomma aegyptium* in *Testudo graeca* in the Sénalba Chergui forest of Djelfa

Overall prevalence rate

The overall prevalence rate observed was 97.33%, indicating an almost generalized infestation of hosts within the studied sample. Such a value points to an almost constant presence of the parasite in the examined tortoises.From an ecological perspective, this very high prevalence suggests that the environmental conditions prevailing in the Djelfa region during the study period were particularly favorable to the maintenance and development of the host–parasite interaction. It also reflects a strong capacity of the parasite to persist and spread within the host population.

3.4 Mean intensity

The mean intensity of infestation was estimated at 7.73 ticks per infested tortoise. In other words, each parasitized host harbored, on average, more than seven ticks, indicating a relatively heavy parasite burden.This high intensity indicates not only that infestation affected almost all the examined tortoises, but also that each infested individual carried a substantial number of parasites. Such a parasite burden may have significant biological consequences for the hosts, particularly on their physiological state, body condition, and potentially their ability to cope with environmental constraints, leading to increased susceptibility to diseases and reduced reproductive success.

3.5 Mean intensity

Overall parasite abundance was estimated at 7.52 ticks per examined individual. This value is very close to the mean intensity of infestation, established at 7.73 ticks per parasitized individual. Such proximity between these two parameters reflects a high level of infestation within the studied population.From an epidemiological and statistical perspective, this slight difference can be explained by the fact that a large proportion of the examined tortoises were infested. Consequently, the mean calculated across all examined individuals (abundance) tends to be very close to that obtained only for infested individuals (mean intensity). This pattern suggests the existence of a high and relatively homogeneous parasite pressure within the analyzed sample.

3.6 Analysis of variance (ANOVA) tests the effects of season, sex, and their interaction on the infestation prevalence of *Hyalomma aegyptium* in *Testudo graeca*.

The factorial ANOVA applied to the prevalence of infestation by *Hyalomma aegyptium* in *Testudo graeca* revealed significant effects of season, sex, and their interaction. A highly significant seasonal effect was observed ($F = 2464.86$; $p < 0.0001$), indicating a marked variation in infestation prevalence across seasons. Likewise, sex showed a significant effect ($F = 78.33$; $p < 0.0001$), highlighting a difference in infestation between male and female individuals. In addition, the interaction between season and sex was significant ($F = 33.71$; $p < 0.0001$), suggesting that the influence of sex on infestation prevalence varies throughout the year. These results reflect a complex relationship between host biology and seasonal variation, which together modulate the dynamics of parasitic infestation.

3.7 Analysis of variance (ANOVA) tests the effects of season, sex, and their interaction on the mean intensity (IM) of *Hyalomma aegyptium* in *Testudo graeca*.

The factorial ANOVA applied to the mean intensity of *Hyalomma aegyptium* infestation in *Testudo graeca* revealed significant effects of season, sex, and their interaction. A highly significant seasonal effect was observed ($F = 134.77$; $p < 0.0001$), indicating a substantial variation in mean intensity across the seasons considered.Seasonality appears to be the predominant factor shaping parasite dynamics. These results demonstrate a major fluctuation in infestation intensity throughout the annual cycle. In spring, not only is there an increase in prevalence but also a substantial rise in individual parasite burden, reflecting heavy infestation.

Likewise, sex showed a significant effect ($F = 81.55$; $p < 0.0001$), highlighting a difference in infestation between male and female individuals. A marked heterogeneity in infestation was recorded between male and female specimens. In agreement with eco-ethological observations in *Testudo graeca*, a significant parasitic bias was noted in the present study, with males exhibiting higher parasite loads.Moreover, the season × sex interaction was significant ($F = 72.99$; $p < 0.0001$), indicating that the influence of sex on mean intensity is not constant throughout the year. This difference, particularly pronounced during the spring reproductive phase, tends to diminish in the autumn, reflecting convergence in host behaviour or ecological niches toward the end of the active season.

3.8 Analysis of variance (ANOVA) tests the effects of season, sex, and their interaction on the abundance of *Hyalomma aegyptium* in *Testudo graeca*.

The analysis of variance (ANOVA) applied to mean abundance revealed highly significant effects for all variables and their interaction ($p < 0.0001$). Season appeared to be the main source of variation ($F = 324.54$, $p < 0.0001$), followed by host sex ($F = 188.78$, $p < 0.0001$) and the season × sex interaction ($F = 171.33$, $p < 0.0001$).

The abundance of ticks in *Testudo graeca* followed a marked seasonal gradient, the magnitude of which differed sharply according to host sex. In spring, a major divergence between the sexes was observed in *Testudo graeca*. While females showed a moderate abundance (1.7), males exhibited the highest parasite abundance (6.3). This spring peak for males suggests increased exposure linked to mate-searching behaviour.In the summer, abundance values became more stable and converged between the two sexes, with moderate means ranging from 1.8 to 1.9.In the fall, the pressure from parasites dropped to its lowest levels, and the numbers for males and females were almost the same (0.3).The non-parallel pattern of the abundance curves confirms a significant synergistic interaction between sex and season. These results demonstrate that the effect of sex on abundance is not uniform: it is maximal in spring and becomes negligible in autumn. This dynamic reflects a close correlation between the phenological cycle of *Hyalomma aegyptium* and the seasonal eco-ethological variations of its tortoise hosts.

3.9 Effects of body size, microhabitat, and their interaction on parasite burden in *Testudo graeca*.

A generalised linear model (GLM) with a Poisson distribution was fitted to evaluate the effects of body size, microhabitat, and their interaction on parasite burden.Overall, the Poisson GLM analysis revealed that the parasite burden of *Hyalomma aegyptium* in *Testudo graeca* varied significantly according to body size and microhabitat. A highly significant positive effect of body size was detected ($\beta = 0.230 \pm 0.042$; $\chi^2 = 29.543$; $p < 0.0001$), indicating an increase in the expected parasite burden with increasing host size. Considering dry microhabitat (drier) as the reference category, microhabitats with litter, shelters, and shrub cover were associated with significantly higher parasite burdens, whereas more open and shaded areas did not show statistically significant differences. Furthermore, the interaction between body size and microhabitat was not globally significant, although an attenuation effect was observed in shelter-type habitats, where the positive influence of body size on parasite burden appeared less pronounced.

3.10 Combined effect of site and season on the distribution of parasite burden.

The chi-square test of independence revealed a significant association between site and season in the distribution of parasite burden ($\chi^2 = 153.394$; df = 6; p < 0.0001). Thus, parasite burden varied significantly according to the site × season combination. The effect size, estimated by Cramer's V, was 0.258, indicating a weak to moderate association.

4. Discussion

Algeria is considered one of the largest countries in North Africa and is characterized by a wide diversity of landscapes, ranging from the Mediterranean coastline to steppe and Saharan zones. Among the terrestrial reptiles inhabiting these environments, the spur-thighed tortoise, *Testudo graeca*, is one of the most emblematic species, particularly in semi-arid regions such as Djelfa. Despite its ecological and heritage importance, this species remains insufficiently studied in several Algerian regions, especially with regard to its local distribution, biology, use of microhabitats, and associated parasitic fauna. Owing to constraints related to climatic conditions, the vast extent of steppe habitats, and the lack of regular field monitoring, knowledge of *Testudo graeca* populations in the Djelfa region remains limited.The present study highlights a very high infestation of *Testudo graeca* by *Hyalomma aegyptium* in the Djelfa forest. The monospecific nature of the observed infestation is consistent with the literature, which identifies *H. aegyptium* as the tick species most closely associated with tortoises of the genus *Testudo* in Mediterranean and steppe environments of the Palearctic region (Široký et al., 2006; Rubel et al., 2025). This exclusive dominance has also been reported in Algeria in populations of *T. graeca*, where all collected ticks belonged to *H. aegyptium* (Aouragh et al., 2020).An overall prevalence of 97.33%, a mean intensity of 7.73 ticks per infested host, and an abundance of 7.52 ticks per examined individual were recorded. These values appear high in comparison with several North African studies. In a semi-arid Algerian zone, Aouragh et al. (2020)

reported a prevalence of 63.6%, a mean intensity of 4.04, and an abundance of 2.57, values markedly lower than those observed here. Likewise, in Tunisia, Gharbi et al. (2015) reported an overall prevalence of 66.19%, whereas in Morocco, Segura et al. (2019) observed in spring a prevalence of 92.5%, an intensity of 6.7, and an abundance of 6.2, values closer to but still lower than those obtained in the present study. By contrast, very high levels were recently documented in Turkish Thrace, with 96.1% prevalence, 24.8 ± 30.6 mean intensity, and 23.8 abundance, showing that infestation levels may become extremely high when ecological conditions are particularly favourable (Kar et al., 2025). These differences among studies may be attributed to bioclimatic contrasts, vegetation structure, sampling season, and the composition of parasitic stages taken into account, which can significantly influence the observed prevalence and intensity of infestations in different regions. Characterized The near-ubiquitous prevalence highlighted in our study suggests that the Djelfa forest provides particularly favourable conditions for maintaining the host–parasite interaction. Authors have also emphasised that habitats with more structured vegetation appear more suitable for tick persistence than open and dry environments. In this context, the high infestation recorded in our study may reflect the combined effect of more complex vegetation, a more favourable microclimate, and a high frequency of contact between tortoises and the infective stages of the parasite.Prevalence, mean intensity, and abundance varied significantly according to season, sex, and the season × sex combination; moreover, males exhibited a spring parasitic peak markedly higher than that of females. This pattern is consistent with studies conducted in Morocco by Segura et al. (2019) and Laghzaoui et al. (2022), which showed that males are often more heavily infested than females. In Segura et al. (2019), this difference was interpreted in light of increased male activity in spring during the reproductive period, which may increase their exposure to ticks. By contrast, this sex bias is not universal, as Aouragh et al. (2020) did not detect any significant difference between males and females in their semi-arid Algerian study area. This divergence among studies indicates that the effect of sex probably depends on the ecological and seasonal context, which your analyses clearly confirm.The positive role of body size on parasite burden is also consistent with the literature. In Tunisia, Gharbi et al. (2015) and, in semi-arid Algeria, Aouragh et al. (2020) also showed a positive relationship between body size and parasite burden.The significant effect of microhabitat highlighted in our study—with higher parasite burdens in the presence of litter, shelters, and shrub cover—is consistent with the idea that more structured and more covered habitats promote host–parasite encounters and the local persistence of ticks. The fact that the body size × microhabitat interaction was not globally significant, despite an attenuated effect in shelters, suggests that host size remains a robust determinant, locally modulated by microhabitat quality.The significant association between site and season in the distribution of parasite burden is consistent with the findings of Segura et al. (2019), who suggested density-dependent effects in host–parasite interactions within Moroccan populations of *Testudo graeca*. In other words, the high infestation level recorded in Djelfa does not appear to result from a single factor but rather from a system in which habitat structure, local tortoise distribution, body size, reproductive behaviour, and seasonality interact.Our results confirm that infestation by *Hyalomma aegyptium* in *Testudo graeca* is not a random process but instead reflects a complex ecological organisation in which season, sex, body size, microhabitat, and spatial variability among sites act simultaneously.

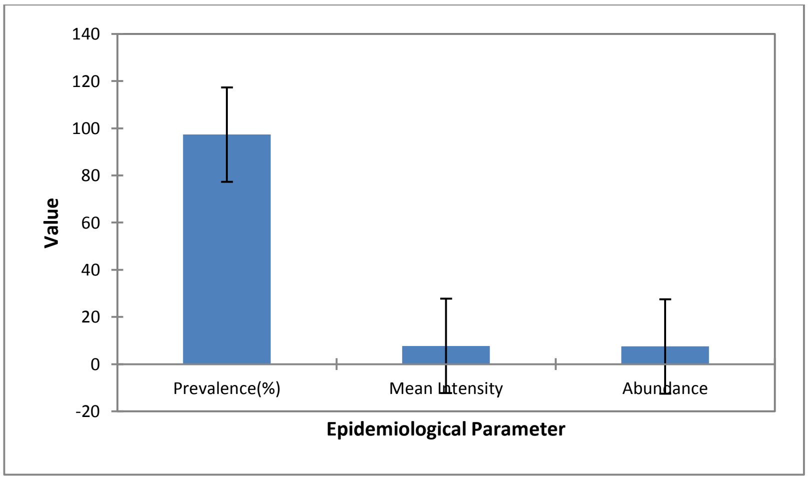

Figure 1. *Epidemiological parameters of infestation by Hyalomma aegyptium in Testudo graeca.*

Table 1. *Results of the factorial ANOVA testing the effects of season, sex, and their interaction on the prevalence of Hyalomma aegyptium in Testudo graeca.*

Source	SS	MS	F	p
Season	15411.410	7705.705	2464.857	< 0.0001
Sex	244.868	244.868	78.327	< 0.0001
Season × Sex	210.790	105.395	33.713	< 0.0001
Residual error	37.515	3.126	—	—
Total	15904.583	—	—	—

Note. SS = sum of squares; MS = mean square; df: season = 2, sex = 1, season × sex = 2, p < 0.05 indicates statistical significance

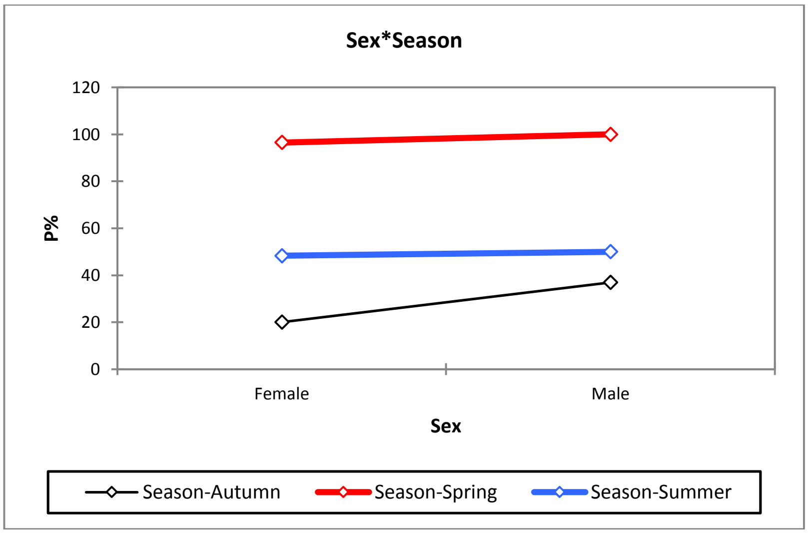

Figure 2: *Variation in infestation prevalence (P%) in Testudo graeca according to host sex and season.*

Table 2. *Results of the factorial ANOVA testing the effects of season, sex, and their interaction on the mean intensity (MI) of Hyalomma aegyptium in Testudo graeca.*

Source	SS	MS	F	p
Season	34.868	17.434	134.774	< 0.0001
Sex	10.549	10.549	81.553	< 0.0001
Season × Sex	18.885	9.443	72.998	< 0.0001
Residual error	1.552	0.129	—	—
Total	65.854	—	—	—

Note. SS = sum of squares; MS = mean square; df: season = 2; sex = 1; season × sex = 2; p < 0.05 indicates statistical significance

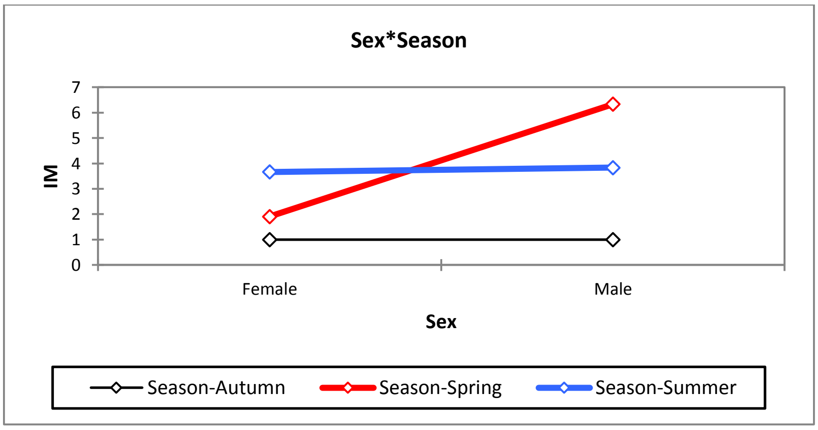

Figure 3: *Variation in mean intensity in Testudo graeca according to host sex and season.*

Table 3. *Results of the factorial ANOVA tests the effects of season, sex, and their interaction on the abundance of Hyalomma aegyptium in Testudo graeca.*

Source	SS	MS	F	p
Season	40.549	20.275	324.538	< 0.0001

Sex	11.794	11.794	188.782	< 0.0001
Season × Sex	21.407	10.703	171.331	< 0.0001
Residual error	0.750	0.062	—	—
Total	74.500	—	—	—

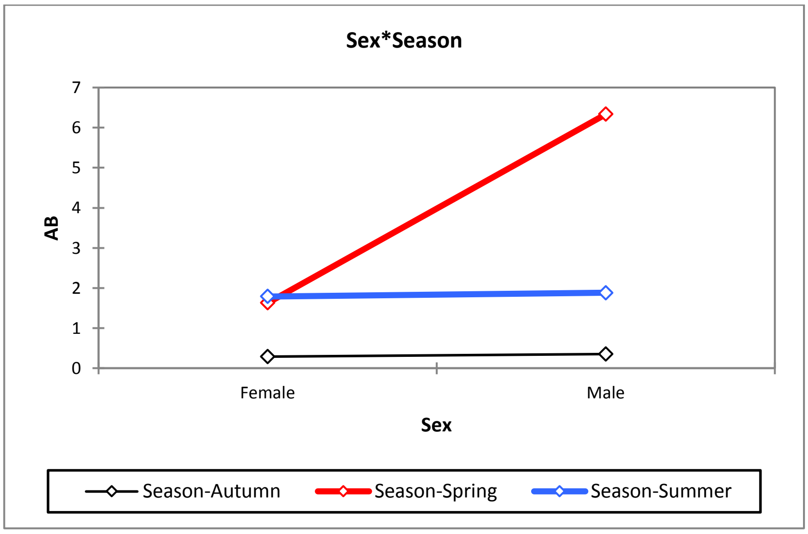

Figure 4: *Variation in abundance in Testudo graeca according to host sex and season.*

Table 4. *Results of the generalised linear model (Poisson distribution) analysing the effects of body size, microhabitat, and their interaction on the parasite burden of Hyalomma aegyptium in Testudo graeca.*

Effect	**Modality / Parameter**	**β**	**SE**	**Test statistic**	**df**	**p value**	**95% CI**
Overall model	Likelihood ratio test	—	—	χ^2 = 403.38	11	< 0.0001	—
Body size	Body size	0.23	0.04	Wald χ^2 = 29.54	1	< 0.0001	[0.15, 0.31]
Microhabitat	Overall effect	—	—	LR χ^2 = 15.35	5	0.009	—
	Drier (reference)	0.00	0.00	—	—	—	—
	More open areas	1.25	1.14	Wald χ^2 = 1.21	1	0.272	[−0.98, 3.48]
	Presence of litter	3.00	1.00	Wald χ^2 = 9.00	1	0.003	[1.04, 4.95]
	Shade	1.93	1.09	Wald χ^2 = 3.14	1	0.076	[−0.21, 4.06]
	Shelters	3.11	1.03	Wald χ^2 = 9.11	1	0.003	[1.09, 5.13]
	Shrub cover	2.77	1.00	Wald χ^2 = 7.74	1	0.005	[0.82, 4.73]
Interaction	Overall effect of body size × microhabitat	—	—	LR χ^2 = 6.99	5	0.221	—
	Body size × more open areas	−0.05	0.06	Wald χ^2 = 0.69	1	0.406	[−0.15, 0.06]
	Body size × presence of litter	−0.09	0.05	Wald χ^2 = 3.84	1	0.050	[−0.19, 0.00]
	Body size × shade	−0.07	0.05	Wald χ^2 = 1.73	1	0.188	[−0.17, 0.03]
	Body size × shelters	−0.12	0.05	Wald χ^2 = 5.32	1	0.021	[−0.21, −0.02]
	Body size × shrub cover	−0.08	0.05	Wald χ^2 = 2.87	1	0.090	[−0.18, 0.01]

Note. β = regression coefficient; SE = standard error; χ^2 = chi-square statistic; LR = likelihood ratio; df = degrees of freedom; CI = confidence interval. Reference category for microhabitat: drier. Significant effects were considered at $p < 0.05$.

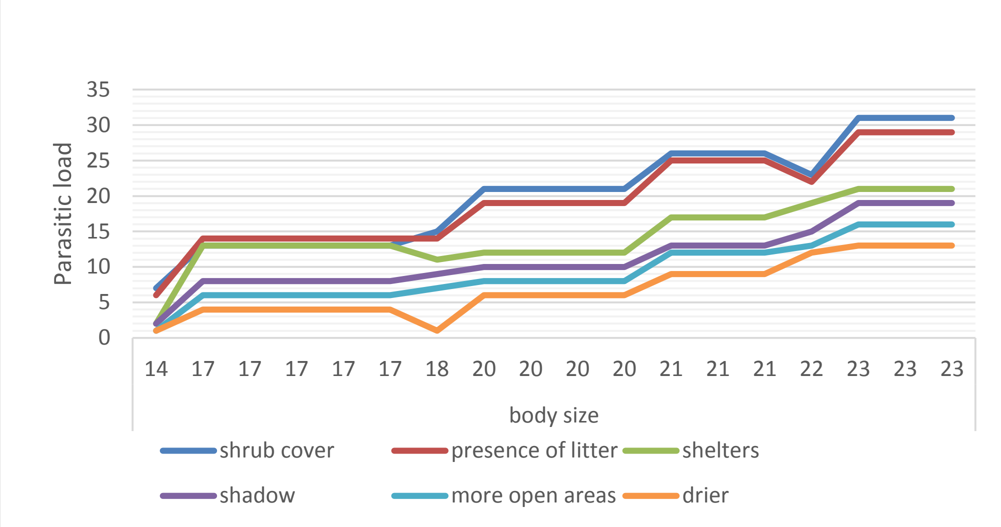

Figure 5: *Effect of body size and microhabitat on parasite burden in Testudo graeca.*

Table 5. *Results of the chi-square test of independence between site and season*.

Compared variables	χ²	df	*p* value	Cramer's V
Site × Season	153.394	6	< 0.0001	0.258

Note *χ² = chi-square statistic; df = degrees of freedom. Cramer's V indicates the strength of association.* Significant effects were considered at *p* < 0.05.

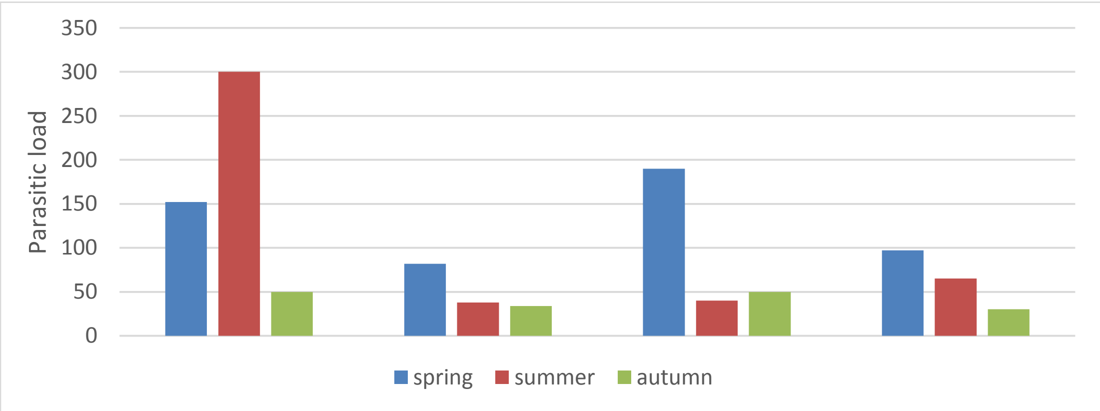

Figure 5: *Combined effect of site and season on the distribution of parasite burden*.

5. Conclusions

This study demonstrates that infestation of Testudo graeca by Hyalomma aegyptium in the Sénalba Chergui forest is both particularly high and significantly influenced by several biological and ecological factors, notably season, sex, body size, and microhabitat. Such a pattern indicates that parasite burden is not randomly distributed within the host population, but rather results from the combined effects of host biological characteristics and local environmental heterogeneity.From an ecological perspective, these findings highlight the determining role of spatial and seasonal variation, as well as microhabitat conditions, in structuring host–parasite interactions. From a conservation standpoint, they emphasize the need to integrate parasitological monitoring into long-term assessments of natural populations of T. graeca as a complementary tool for evaluating population health status, habitat quality, and ecological vulnerability.

Author Statements:

- **Ethical approval:** The conducted research is not related to either human or animal use.
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- **Use of AI Tools:** The author(s) declare that no generative AI or AI-assisted technologies were used in the writing process of this manuscript.

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